Article

Ancient *Plasmodium* genomes shed light on the history of human malaria

https://doi.org/10.1038/s41586-024-07546-2

Received: 10 September 2023

Accepted: 9 May 2024

Published online: 12 June 2024

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Malaria-causing protozoa of the genus *Plasmodium* have exerted one of the strongest selective pressures on the human genome, and resistance alleles provide biomolecular footprints that outline the historical reach of these species[1]. Nevertheless, debate persists over when and how malaria parasites emerged as human pathogens and spread around the globe[1,2]. To address these questions, we generated high-coverage ancient mitochondrial and nuclear genome-wide data from *P. falciparum*, *P. vivax* and *P. malariae* from 16 countries spanning around 5,500 years of human history. We identified *P. vivax* and *P. falciparum* across geographically disparate regions of Eurasia from as early as the fourth and first millennia BCE, respectively; for *P. vivax*, this evidence pre-dates textual references by several millennia[3]. Genomic analysis supports distinct disease histories for *P. falciparum* and *P. vivax* in the Americas: similarities between now-eliminated European and peri-contact South American strains indicate that European colonizers were the source of American *P. vivax*, whereas the trans-Atlantic slave trade probably introduced *P. falciparum* into the Americas. Our data underscore the role of cross-cultural contacts in the dissemination of malaria, laying the biomolecular foundation for future palaeo-epidemiological research into the impact of *Plasmodium* parasites on human history. Finally, our unexpected discovery of *P. falciparum* in the high-altitude Himalayas provides a rare case study in which individual mobility can be inferred from infection status, adding to our knowledge of cross-cultural connectivity in the region nearly three millennia ago.

Malaria is a vector-borne disease caused by protozoa in the genus *Plasmodium* and is transmitted by female anopheline mosquitoes[4]. It is a major cause of human morbidity and mortality, with an estimated 240 million cases and more than 600,000 fatalities in 2020 (ref. 5). Beyond its current health impact, malaria has profoundly influenced human evolution, exerting one of the strongest identified selective pressures on the human genome. Congenital haematological conditions, including sickle-cell disease, G6PD deficiency and thalassaemia, have persisted because they confer partial resistance to malaria, indicating a long-term relationship between the pathogen and human populations[6].

Of the five primary human-infecting *Plasmodium* species, *P. falciparum* and *P. vivax* account for the vast majority of malaria disease burden today, whereas *P. malariae*, *P. ovale wallikeri* and *P. ovale curtisi* are less common and cause milder symptoms[4]. Previous research indicates that *P. falciparum* emerged through zoonosis from gorillas in sub-Saharan Africa[7]. Date estimates for the most recent common ancestor of extant *P. falciparum* strains range from less than 10,000 to 450,000 years ago[8–10].

The emergence of *P. vivax* is generally considered to pre-date that of *P. falciparum*, but its evolutionary origins are less well understood. Early mitochondrial analyses supported an origin in Southeast Asia, placing *P. vivax* in a clade of *Plasmodium* species infecting macaques and other Southeast-Asian primates[11,12]. Analyses based on nuclear data, including phylogenies and patterns of nucleotide diversity, have provided further support for an Asian origin[13]. However, parasites of the African great apes, notably *P. carteri* and *P. vivax*-like, are now thought to constitute the closest relatives of *P. vivax*[10,14,15]. Together with the near-fixation of the Duffy-negative allele in many human groups in sub-Saharan Africa, this provides strong support for an African origin for *P. vivax*[1]. The Duffy antigen, encoded by the *FY* locus, facilitates *P. vivax* erythrocyte invasion, and individuals homozygous for the Duffy-negative allele were once considered completely immune to *P. vivax* malaria[1,6]. Accumulating evidence demonstrates that populations with high rates of Duffy negativity can maintain low levels of *P. vivax* transmission, and the phenotype seems to reduce the efficiency of erythrocyte invasion and provide protection against blood-stage infection[16]. Thus, proponents of the African-origin hypothesis argue that a long history of selection pressure exerted by *P. vivax* drove increases in the Duffy-negative phenotype, making these populations less susceptible to *P. vivax* infection today. Interestingly, some human groups in Papua New Guinea have a Duffy null allele that seems to have arisen through an independent mutation. Indeed, the low frequency and long haplotype associated with the Papua New Guinea variant support more recent positive selection in people living in Oceania than in those in sub-Saharan Africa[17].

As well as the evolutionary constraints, variation in pathogenesis between *P. vivax* and *P. falciparum* contributes to their distinct geographical distributions and ecologies. Because of its higher virulence, morbidity and mortality, *P. falciparum* requires a larger population of susceptible hosts to sustain transmission. Consequently, some researchers have theorized that hunter-gatherer population densities were probably too low to support the emergence of *P. falciparum*, which instead may have proliferated with the development of agriculture in sub-Saharan Africa[1]. Climate also poses distinct constraints on the

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ranges of these two species, with *P. vivax* able to survive and develop at lower temperatures than *P. falciparum*[18,19]. Finally, *P. vivax* forms hypnozoites in its dormant hepatic stage, and reactivation months or even years after an initial infection can re-initiate the *Plasmodium* life cycle, enabling further transmission[4]. Hypnozoites enable *P. vivax* to overwinter in the human host when low temperatures limit vector activity. Combined with its greater tolerance for cold temperatures, this capacity enables *P. vivax* to survive in temperate regions, whereas *P. falciparum* is generally restricted to tropical and subtropical zones[1].

Because *Plasmodium* species are obligate intracellular pathogens, their contemporary distributions reflect patterns of human mobility, as well as the evolutionary, physiological and ecological constraints acting on the parasite, human host and mosquito vector. However, relatively little is known about the timing and routes by which *Plasmodium* spp. spread around the globe. In the palaeopathological literature, cribra orbitalia and porotic hyperostosis have been considered to be indicators of severe malarial anaemia[20,21]. However, their presence should be interpreted with caution because these skeletal lesions are not pathognomonic for the identification of malaria cases in the archaeological record[22,23], and the two conditions probably have different underlying aetiologies[24,25]. Recurrent fevers are described in Vedic and Brahmanic texts from the first millennium BCE, and Hippocratic texts from the late fifth or early fourth century BCE provide the first unambiguous references to malaria in the Mediterranean world[1,3]. However, retrospective diagnosis of malaria poses considerable challenges, and many time periods and regions are missing from the historical record[26]. Although written sources and congenital haematological conditions provide indirect evidence of the historical range of malaria, uncertainty persists over which species contributed to selective processes in specific regions, as well as how the selective dynamics played out over time[1,2].

Tracing the history of *Plasmodium* spp. in the Americas is of particular interest, given the limited number of transoceanic contacts that may have facilitated transmission. *P. falciparum* is likely to have reached the Americas with colonizers from Mediterranean Europe or as a result of the trans-Atlantic slave trade, but the potential pre-contact origin of American *P. vivax* is still debated[27]. Some scholars suggest that *P. vivax* reached the American continent with its first human inhabitants, and cite as evidence both its high nucleotide diversity and the presence of divergent mitochondrial lineages in American parasite populations[28]. Others argue that American *P. vivax* may derive from pre-colonial-era contacts with Oceanian seafarers[27]. Finally, *P. vivax*, as well as *P. falciparum* and many other Eurasian pathogens, may have reached the Americas during the European colonial era[28–30]. A contact-era introduction of *Plasmodium* spp. is consistent with the absence of malaria-resistance alleles in the Indigenous peoples of the Americas[31]. Further support for this hypothesis comes from analyses of the only historical European *P. vivax* genomic dataset available to date, which derives from a 1944 blood slide from Spain's Ebro Delta. Analysis of nuclear single-nucleotide polymorphism (SNP) data places Ebro1944 close to contemporary South and Central American *P. vivax* strains[30].

The ability to retrieve ancient bacterial and viral DNA preserved in human skeletal material is providing a fuller picture of the evolution, origins and global dissemination of historically important pathogens[32]. However, attempts to retrieve ancient DNA from *Plasmodium* spp. have until now had limited success[33]. Apart from Ebro1944 (refs. 30,34,35), the available ancient *Plasmodium* datasets have so far been restricted to two partial mitochondrial genomes from southern Italy dating to the first and second century CE[36]. Here we identify *P. falciparum*, *P. vivax* and *P. malariae* infections in 36 ancient individuals from 16 countries spanning 5,500 years of human history from the Neolithic to the modern era. Using two new in-solution hybridization capture bait sets, we generate high-coverage ancient *Plasmodium* mitochondrial genomes and genome-wide nuclear data, which demonstrate that the European expansion of *P. vivax* greatly pre-dates evidence from written sources. Genomic data from now-eliminated European *P. falciparum* and *P. vivax* strains provide an unprecedented opportunity to explore gaps in the genomic diversity of modern *Plasmodium* populations, enabling a fuller picture of the origins and transmission routes of human malaria parasites. Finally, contextualizing ancient genomic data from *P. falciparum* and *P. vivax* alongside archaeological information and human population genetics reveals the critical role of human mobility in the spread of malaria in past populations.

Ancient *Plasmodium* spp. data generation

To identify ancient malaria cases, we performed a metagenomic analysis of previously produced shotgun-sequenced libraries from more than 10,000 ancient individuals (Methods). Ancient DNA libraries found to possess traces of *Plasmodium* DNA were enriched using two new hybridization capture reagents targeting the mitochondrial and nuclear genomes of *Plasmodium* spp. In total, we identified 36 malaria cases, comprising 10 *P. falciparum* infections, 2 cases of *P. malariae* and 21 *P. vivax* infections, along with 2 individuals co-infected with *P. falciparum* and *P. malariae* as well as 1 *P. vivax*–*P. falciparum* co-infection (Fig. 1, Supplementary Table 1 and Supplementary Note 1). We analysed these ancient mitochondrial and nuclear datasets alongside modern *Plasmodium* data and published shotgun reads from the Ebro1944 blood slide[30,34,35,37,38].

Mitochondrial capture allowed for the reconstruction of full genomes from 13 *P. falciparum* strains with mean coverage ranging from 1.1× to 118.3×, 6 *P. vivax* strains with mean coverage of 3.0× to 94.3× and 4 *P. malariae* strains with 1.1× to 80.4× mean coverage (Extended Data Fig. 1, Supplementary Table 2 and Supplementary Note 2). To further explore the population structure of ancient *P. vivax* and *P. falciparum*, we genotyped our ancient nuclear-capture datasets at high-quality biallelic SNP positions ascertained in modern datasets published by the MalariaGEN *P. vivax* Genome Variation Project and the MalariaGEN *P. falciparum* Community Project, respectively[37,38] (Extended Data Fig. 2). For *P. falciparum*, we merged data from 1,227 modern and 8 ancient strains genotyped at 106,179 segregating SNP positions, and for *P. vivax* our final dataset contained 906 modern and 23 ancient strains genotyped at 419,387 segregating SNP positions. The coverage of our ancient samples ranged from 541 to 19,525 SNPs for *P. falciparum* (median of 1,068 SNPs) and from 721 to 208,344 SNPs for *P. vivax* (median of 2,153.5 SNPs) (Supplementary Table 3 and Supplementary Note 3).

Early presence of malaria in Eurasia

Previous attempts to outline the past distribution of *Plasmodium* spp. have relied on textual references that provided evidence for *P. falciparum* in the Greek world as early as around 400 BCE and in South Asia from the early first millennium BCE[3]. Our ancient *P. falciparum* data from the Himalayan site of Chokhopani (a calibrated (cal) date of around 804–765 cal BCE[39]; Supplementary Note 1.1.3) and the Central European Iron Age site of Göttlesbrunn (around 350–250 BCE; Supplementary Note 1.1.6) complement these textual references, shedding light on the role of mobility and trade in transmitting malaria beyond historically documented centres of endemicity (Fig. 1). Chokhopani is situated in a high transverse Himalayan valley around 2,800 m above sea level, but grave goods indicate that there were trade connections with the Indian subcontinent that may have facilitated the spread of malaria into the highlands[39]. Similarly, Göttlesbrunn was part of some trans-regional exchange networks, as evidenced by the archaeological record[40], and historically attested conflicts brought Late Iron Age Central European populations into potentially malarious regions of the Mediterranean and the Balkans[41].

Biomolecular data also provide firm evidence for the widespread impact of *P. vivax* on prehistoric European populations. We have identified three *P. vivax*-infected individuals dating from the third or fourth millennium BCE, including a Middle Neolithic Baalberge individual from

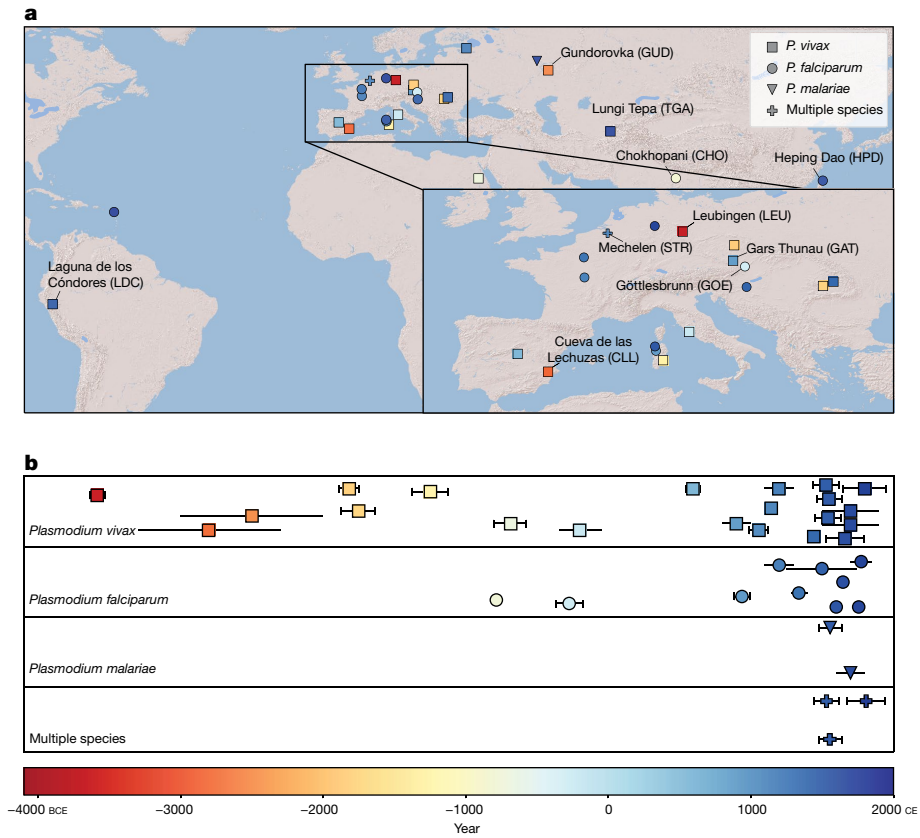

Fig. 1 | Spatial and temporal distribution of *Plasmodium*-positive ancient individuals. a, Archaeological sites with malaria-positive ancient individuals. Site colour reflects the date-range midpoint for the infected individual(s). Names and abbreviations are included for sites discussed in the main text. Map produced using Cartopy (v.0.20.3, https://github.com/SciTools/cartopy/tree/v0.20.3), Natural Earth (naturalearthdata.com) and World Shaded Relief map (Esri). **b**, Temporal distribution of $n = 36$ malaria-positive ancient individuals. Points reflect date-range midpoints; error bars indicate uncertainty inferred from either archaeological context (uncapped error bars) or radiocarbon dating (capped error bars, calibrated calendar ages, 2σ range) (Supplementary Table 1).

Leubingen, Germany (3,637–3,528 cal BCE; Supplementary Note 1.2.7), a Chalcolithic individual from Cueva de las Lechuzas, Spain (3,300–2,300 BCE[42]; Supplementary Note 1.2.1) and an Eneolithic individual from Gundorovka in Russia (turn of the fourth to third millennium BCE[43]; Supplementary Note 1.2.5) (Fig. 1). Finding *P. vivax* in 3 ecologically disparate sites more than 5,000 km apart indicates that this species probably affected large portions of Europe by the fourth millennium BCE, predating the earliest textual evidence for malaria by several thousand years[1,3]. Evidence for *P. vivax* infection at Gundorovka is especially noteworthy: although the site was used for a period spanning the Neolithic–Eneolithic through the Middle–Late Bronze Age and Early Iron Age, the individual analysed here has been contextually dated to the Eneolithic period[43]. Our findings underscore the need for further sampling to fully elucidate the capacity of low-density transitional hunter-gatherer groups to sustain malaria transmission before the full-scale adoption of agriculture and sedentism.

P. vivax population genetics

Consistent with previous studies, analysis of nuclear SNP data revealed a strong phylogeographic structure in modern *P. vivax* populations[37]. In a principal component analysis (PCA), strains from proximal regions formed distinct clusters, and the first two principal components (PCs) captured a large proportion of this genetic variation (9.47% and 5.54% for PC1 and PC2, respectively), defining three main clusters: (1) Africa, Western Asia and Latin America (South and Central America); (2) East and Southeast Asia; and (3) Oceania (Fig. 2). Our ancient *P. vivax* dataset includes six strains with nuclear SNP coverage levels suitable for population genetic analysis (Supplementary Note 4): STR105 and STR185 from the medieval/early modern cemetery of St. Rombout in Mechelen, Belgium (Supplementary Note 1.4.1); GAT004 from the early medieval Austrian site Gars Thunau (Supplementary Note 1.2.3); the previously published Ebro1944 dataset[30,34,35]; LDC020, dated to the peri-contact period (1437–1617 cal CE) from the Chachapoya site of Laguna de los Cóndores, Peru (Supplementary Note 1.2.6); and TGA007 from the late medieval/early modern period in southern Uzbekistan (Supplementary Note 1.2.8). Our data provide an opportunity to assess diversity in European *P. vivax* populations spanning the colonial era. All higher-coverage European strains fall in a tight cluster in PCA space, indicating the presence of a single, broadly distributed European population exhibiting genetic continuity from the medieval to the modern period (Fig. 2). Assessment of our ancient samples using PCA, ADMIXTURE and F_4 statistics also provided evidence for stability in *P. vivax* population structure over time (Extended Data Figs. 3–6, Supplementary Table 4 and Supplementary Note 5). Falling within the diversity of modern Latin American strains, LDC020 exhibits a closer affinity to modern Peruvian *P. vivax* than to modern strains from Colombia, Brazil and Central America (Supplementary Table 5 and Supplementary Note 6). Similarly, PCA places TGA007 adjacent to modern Western Asian populations sampled from Afghanistan, India, Iran and Sri Lanka, and adjacent to and shifted towards two admixed strains from modern Bhutan. Finally, low-coverage samples from Uzbekistan and Pharaonic Egypt also show relatedness to geographically proximal modern populations (Extended Data Fig. 3 and Supplementary Note 5). Such affinities in strains sampled centuries apart may reflect long-term persistence of endemic foci in Latin America and western/southern Asia, an observation that is consistent with the refractory nature of *P. vivax* populations to contemporary eradication campaigns[44].

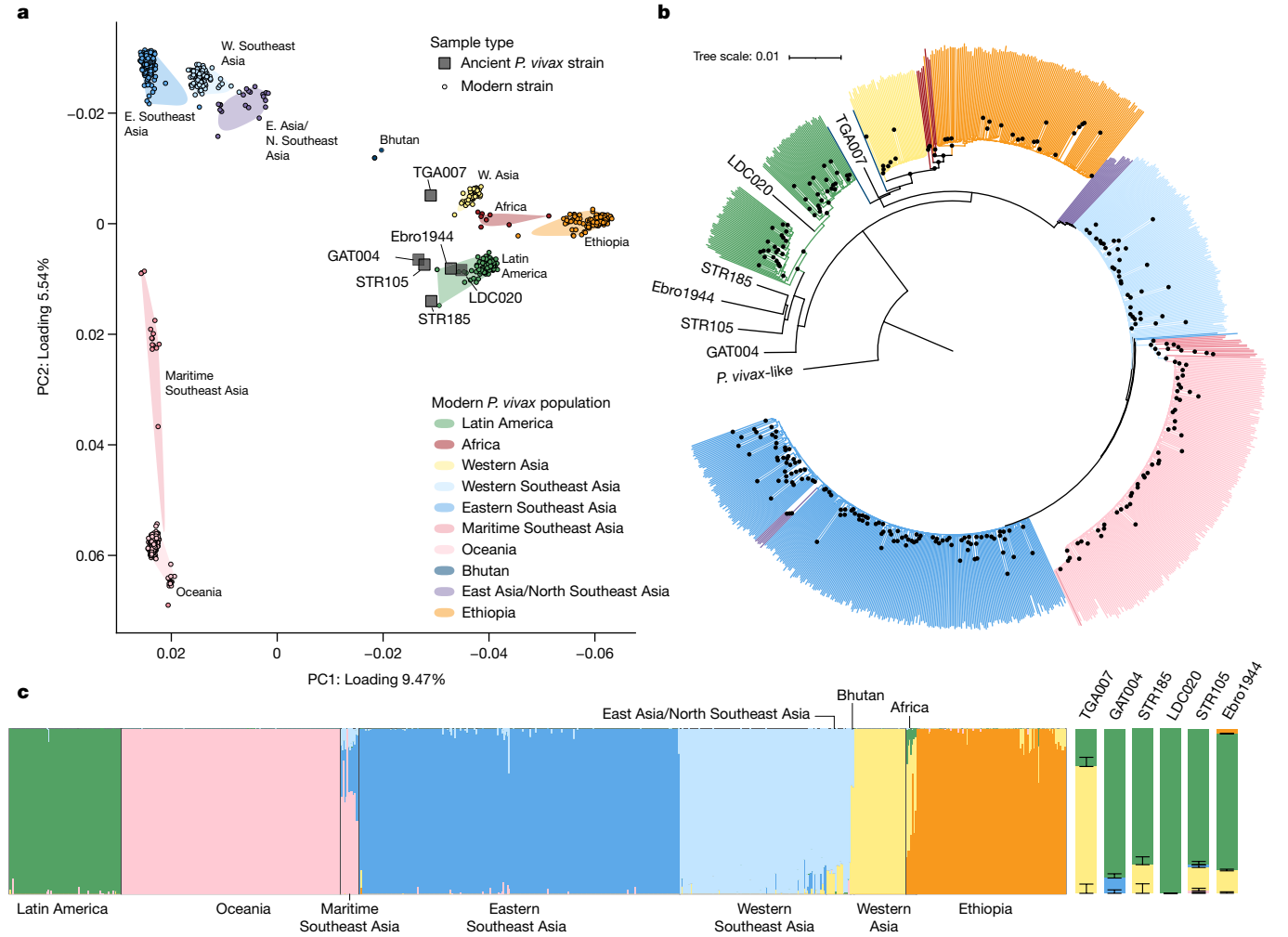

Fig. 2 | *P. vivax* population genetics. a, Ancient *P. vivax* strains with more than 5,000 SNPs covered (grey squares). Ancient data are projected onto modern *P. vivax* strains (small points) genotyped by the MalariaGEN *P. vivax* Genome Variation Project[37]. Shaded regions delimit the spread of modern *P. vivax* populations in PCA space. **b**, Neighbour-joining phylogeny including ancient and modern *P. vivax* strains. Branches are coloured by geographic origin, as in **a**, black points reflect nodes receiving support values greater than or equal to 0.9 (100 bootstrap replicates). **c**, Unsupervised ADMIXTURE analysis of modern *P. vivax* populations using $K = 6$ ancestry sources (left), and supervised ADMIXTURE analysis of high-coverage (more than 5,000 SNPs) ancient *P. vivax* strains (right). Ancient strains were modelled as mixtures of $K = 6$ ancestral sources maximized in the following modern populations: Latin America, Oceania, maritime Southeast Asia, eastern Southeast Asia, western Southeast Asia, western Asia and Ethiopia. Error bars reflect uncertainty in mean individual admixture proportions (standard errors, 300 bootstrap replicates).

P. falciparum population genetics

As for *P. vivax*, analysis of nuclear SNP data revealed considerable phylogeographic structure in modern *P. falciparum* populations, with PCA defining the following three clusters: (1) Africa and South America; (2) South and Southeast Asia; and (3) Oceania (Fig. 3). As previously observed, modern *P. falciparum* exhibits lower genetic diversity than *P. vivax*. In a global set of 1,227 *P. falciparum* samples published by the MalariaGEN project[38], we observed only 106,179 high-quality biallelic segregating SNPs, compared with 419,387 positions in a set of 906 *P. vivax* strains. Furthermore, as a consequence of the organism's higher AT skew and lower complexity, our probe set spans a smaller proportion of the *P. falciparum* nuclear genome (Extended Data Fig. 7), meaning that *P. falciparum* strains generally attain lower coverage in our ancient dataset. Nevertheless, 3 samples exhibit coverage levels of more than 10,000 SNPs: CHO001 from the first millennium BCE Himalayan site of Chokhopani (Supplementary Note 1.1.3); HPD007 from the seventeenth-century Spanish colonial outpost of Heping Dao off the coast of Taiwan (Supplementary Note 1.1.7); and Ebro1944 (refs. 30,34,35). Interestingly, these genomes, along with other lower-coverage European strains, fall into a gap in PCA space and are modelled as complex population mixtures in supervised ADMIXTURE analysis (Fig. 3, Extended Data Figs. 4, 5 and 8 and Supplementary Note 7). This observation indicates that our ancient strains cannot be clearly assigned to one currently sampled modern *P. falciparum* population, possibly reflecting sampling biases in modern comparative datasets. Apart from Ebro1944, our ancient dataset provides a first glimpse into the genetics of now-eliminated European *P. falciparum* populations. Furthermore, despite constituting an important centre of *P. falciparum* endemicity, the MalariaGEN *P. falciparum* Community Project Pf6 data release lacks genotype data from India. We attempted to address this problem by analysing our data alongside published shotgun-sequencing data from five *P. falciparum* strains retrieved from hospitalized patients in Goa[45]. Indeed, based on PCA, F_3 statistics and ChromoPainter/fineSTRUCTURE, the Ebro1944 strain showed a higher affinity to these Indian genomes than to other modern populations (Fig. 3, Extended Data Fig. 8 and Supplementary Note 7). This observation may reflect links between European and South Asian *P. falciparum* populations, as previously proposed[34], but more sampling is needed to further support this hypothesis and clarify the population affinities of our ancient strains.

Alternative histories in the Americas

In this study, we generated high-coverage *P. vivax* genome-wide nuclear and mitochondrial data from a peri-contact South American individual from the site of Laguna de los Cóndores in Peru (LDC020). Associated with the Chachapoya culture and radiocarbon dated to between 1437 and 1617 cal CE, analysis of human genome-wide data indicated an individual of Indigenous ancestry with no evidence of European admixture (Extended Data Fig. 9 and Supplementary Note 8). The LDC020 *P. vivax* strain overlaps with modern South American populations in PCA and can be modelled as deriving 100% of its genetic ancestry from American-related populations in supervised ADMIXTURE analysis (200 bootstrap replicates; Fig. 2). F_3 statistics indicate that LDC020 is related more closely to Latin America than to any other modern *P. vivax* population (Extended Data Fig. 3 and Supplementary Note 5), and F_4 statistics demonstrate that LDC020 shows excess affinity with modern Peruvian *P. vivax* populations compared with modern strains from Colombia, Brazil and Central America (Supplementary Table 5 and Supplementary Note 6). Together, this evidence suggests that LDC020 is closely related to the ancestors of *P. vivax* circulating in the Americas today, and the genetic links between modern and ancient Peruvian *P. vivax* support the early establishment and long-term maintenance of an endemic focus in the region.

Interestingly, both PCA and F_4 statistics indicate that ancient European *P. vivax* strains are also related more closely to modern and ancient Latin American strains than to any other modern population (Fig. 2, Extended Data Fig. 3, Supplementary Table 4 and Supplementary Note 5). A neighbour-joining phylogeny constructed using genome-wide SNP data places the ancient European *P. vivax* strains basal to a clade formed by LDC020 and modern Latin American lineages (Fig. 2). Together, the close relationship between pre-elimination European populations, modern American *P. vivax* and LDC020 supports the introduction of *P. vivax* from European populations to the Americas during the contact period. A non-African source for American *P. vivax* is also consistent with the low frequency of *P. vivax* in regions of sub-Saharan Africa with high rates of Duffy negativity. Overall, this evidence for a close genetic link between American and extirpated European strains indicates that *P. vivax* was probably absent in the Americas before the colonial period, although we cannot exclude the possibility of a replacement of pre-contact *P. vivax* variation after the introduction of strains from Europe.

Although our dataset lacks ancient Latin American *P. falciparum* strains, it sheds light on the relatedness between modern lineages and ancient European *P. falciparum* strains spanning the contact era. As noted above, ancient European *P. falciparum* strains fall in a distinct region in PCA space that does not overlap with currently sampled modern populations. On the contrary, all modern South American *P. falciparum* strains sequenced to date form a tight cluster closely related to strains from West, Central and East Africa. Analyses using F_4 statistics further support the close relationship between South American and African *P. falciparum*, although a minor contribution from European lineages cannot be excluded (Supplementary Table 6 and Supplementary Note 7). Together with the high prevalence of *P. falciparum* in sub-Saharan Africa today, our population genetic analysis supports the transmission of this species to the Americas as a result of the trans-Atlantic slave trade[27,46].

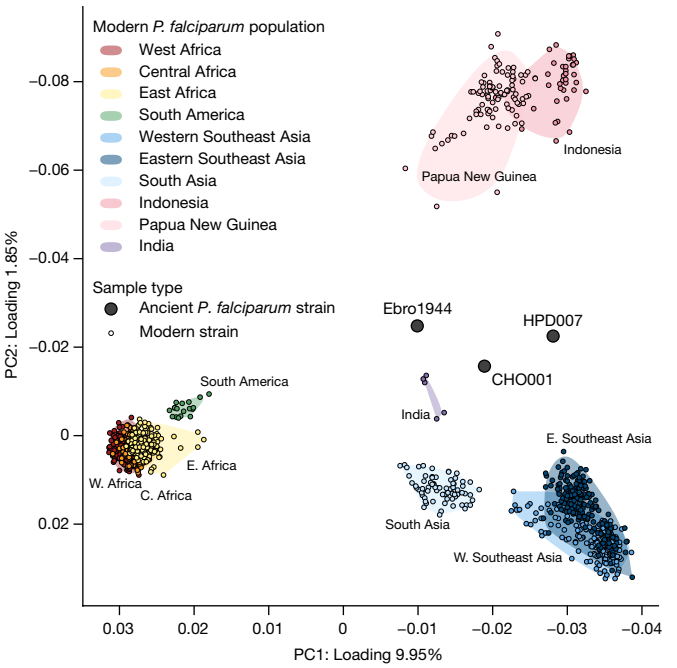

Fig. 3 | *P. falciparum* PCA. Ancient *P. falciparum* strains with more than 10,000 SNPs covered (labelled circles). Ancient strains are projected onto the diversity of modern *P. falciparum* genomes published by the MalariaGEN *P. falciparum* Community Project[38]. Modern strains are shown as small points, and the shaded regions delimit the distribution of modern *P. falciparum* populations in PCA space.

Human mobility and malaria transmission

The unexpected recovery of *P. falciparum* and *P. vivax* genomes from individuals at the high-altitude Himalayan site of Chokhopani (2,800 m above sea level) and the Andean site of Laguna de los Cóndores (2,860 m above sea level) underscores the role of human mobility in spreading malaria. In general, elevation limits endogenous malaria transmission. The colder and potentially drier conditions associated with high altitudes may be unsuitable for mosquito survival and reproduction, and temperatures below species-specific thresholds inhibit the development of *Plasmodium* parasites inside mosquito vectors[47]. Precise altitudinal limits on malaria endemicity depend on a variety of factors, including latitude, microclimate, landscape modification and the *Plasmodium* and *Anopheles* species present, and boundaries may shift dynamically in response to changes in climate and/or the local environment. Although the complex ecology of *Plasmodium* transmission complicates attempts to reconstruct past endemic ranges, modern epidemiological and climatological data are sufficient to render malaria transmission at Chokhopani highly unlikely (Supplementary Note 9).

Instead, we hypothesize that malaria cases at highland sites reflect transregional transmission from lowland areas capable of sustaining endemic foci. Situated in a high transverse Himalayan valley linking the Tibetan Plateau with southern lowland areas, the region surrounding Chokhopani may have served as an epicentre of trade and exchange in the first millennium BCE. Consisting of a series of shaft tombs built into a riverside cliff, the site contained three burial chambers containing the remains of at least 21 individuals, as well as copper grave goods similar to those produced in the Indian subcontinent[39,48,49] (Supplementary Note 1.1.3). Owing to the commingled nature of the remains, skeletal material from CHO001 was limited to the permanent molar yielding *P. falciparum* DNA. Previous studies found that the genetically male individual CHO001 possessed alleles associated with high-altitude adaptation and exhibited ancestry similar to that of present-day Tibetans[50] (Supplementary Note 1.1.3). Notably, individuals from Chokhopani also have a minor lowland South Asian ancestry component that is absent in other prehistoric sites in Upper Mustang; this finding further supports the connection between Chokhopani and lowland South Asian regions, although the admixture event probably occurred around 500–1,000 years before the *P. falciparum*-infected individual identified here lived[50]. Finally, the relatively short overland distances between Chokhopani and regions of contemporary malaria endemicity in the Nepalese and

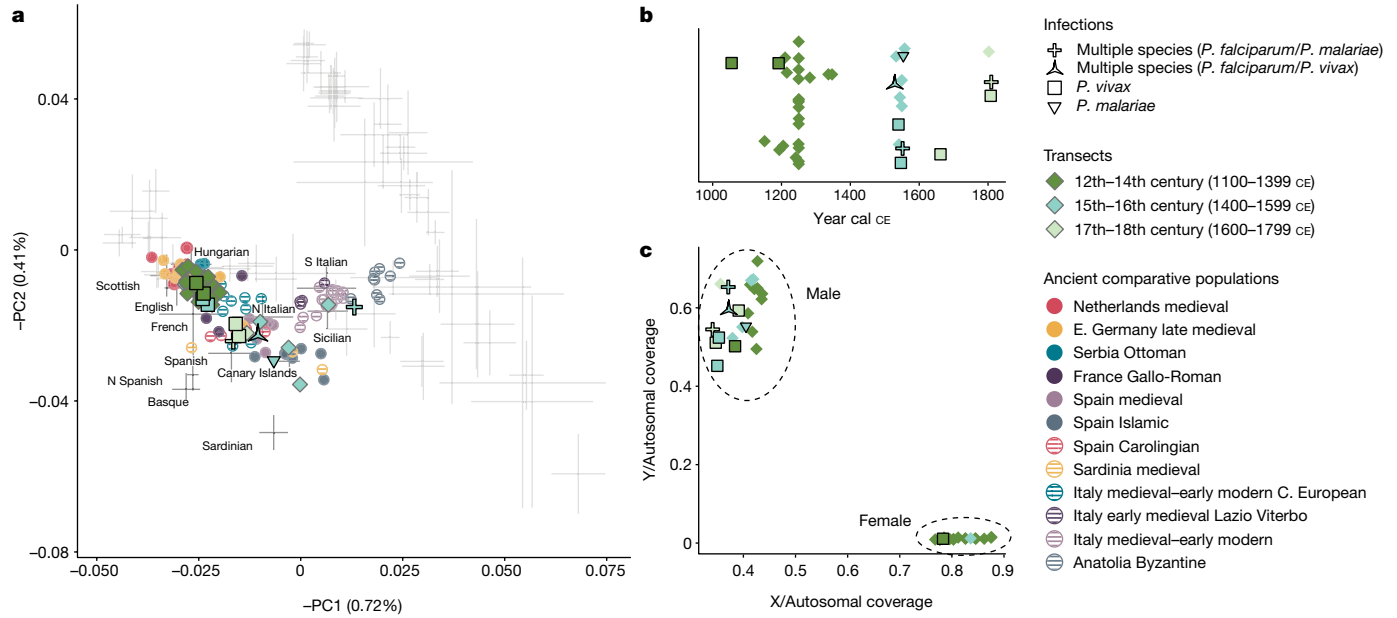

Fig. 4 | Shift in human ancestry and malaria infectivity at Mechelen, Belgium. **a**, PCA showing both infected and uninfected ancient individuals projected onto the diversity of modern Western Eurasian populations. Marker type indicates infection status and colouration reflects the temporal layer of ancient individuals. Selected ancient populations are shown as coloured circles for comparative purposes. **b**, Chronology of individuals yielding human and/or *Plasmodium* genome-wide data. Individuals are classified as deriving from the twelfth-to-fourteenth centuries CE, the fifteenth-to-sixteenth centuries or the seventeenth-to-eighteenth centuries on the basis of their calibrated radiocarbon dates or available archaeological context. **c**, Relative coverage on the X and Y chromosomes used for sex determination.

Indian Terai underscore the likely role of individual mobility in spreading *P. falciparum* into the Himalayan highlands[51]. Taken together, our discovery of a *P. falciparum* infection in the Chokhopani individual adds to a growing body of evidence for cross-cultural connectivity, even in this remote Himalayan region. Given the genetic links between CHO001 and other modern and ancient high-altitude populations, we suggest that this individual lived locally and contracted malaria while travelling to or from an adjacent endemic region. However, we cannot exclude the possibility that CHO001 was a non-local individual who travelled to Chokhopani from a nearby malarious area. Overall, we highlight CHO001 as a rare case study in which aspects of an individual's mobility can be inferred from their infectious-disease status, which is an important finding given the limited information that could be drawn from the fragmented skeletal material associated with this individual.

Long-distance exchange may also have facilitated the spread of *P. vivax* into the vicinity of Laguna de los Cóndores (LDC; Supplementary Note 9). The Chachapoya cultural region, including LDC, is in the subtropical forest of the eastern Andean slopes, providing an appropriate environment for mosquitos to thrive. Despite the remote location of the region today, archaeological evidence suggests that the Chachapoya cultural region was home to many pre-colonial societies and served as an intersection of cultural connectivity and exchange for communities across the Andes to the Amazon Basin[52]. Indeed, the discovery of Amazonian feathered head-dresses and preserved lowland-animal pelts at LDC attests to exchange networks with areas of modern malaria endemicity[52]. Furthermore, the Spanish invasion and conquest is known to be one of the main factors contributing to the spread of infectious diseases throughout the Americas, leading to drastic population declines for many Indigenous groups that some suggest were as large as 90% (ref. 53). In some regions, introduced pathogens spread rapidly along existing networks of connectivity, decimating local Indigenous populations even before the arrival of colonial military forces[53,54]. Later, the Spanish displaced large numbers of Indigenous inhabitants, who were conscripted to fight against the Inca or to explore the Amazon[55]. Together, warfare, Spanish colonization and other socio-political upheavals may have accelerated the spread of malaria in the Andean hinterlands early in the colonial era.

The identification of ten malaria-infected individuals from the cemetery of St. Rombout in Mechelen, Belgium, further illustrates the capacity of warfare and individual mobilization to drive malaria transmission (Supplementary Note 1.4.1). Situated directly adjacent to the first permanent military hospital in early modern Europe, which was in use from 1567 to 1715 CE, the cemetery may have served as a burial place for soldiers in the Habsburg Army of Flanders[56,57]. Excavations of the cemetery unearthed the remains of 4,158 articulated individuals from 3 main layers, approximately dated to the twelfth-to-fourteenth centuries CE, the fifteenth-to-sixteenth centuries CE and the seventeenth-to-eighteenth centuries CE; the last 2 phases overlap with the time the hospital was in use[57,58]. Interestingly, our pathogenomic and human population genetic analyses of 40 individuals from Mechelen support the hypothesis that the cemetery contained at least 2 distinct subgroups. Studying 25 individuals dated to the earliest phase (twelfth-to-fourteenth centuries CE) reveals an approximately equal sex ratio, and these individuals formed a tight cluster in PCA overlapping with geographically proximal modern populations for which genotype data are available (including French, English, Scottish and Hungarian), as well as late-medieval Germany and the Netherlands[59] (Fig. 4 and Supplementary Table 7). Consistent with this signature of central/northern European ancestry, both of the malaria infections in the early transect were caused by *P. vivax*, a species adapted to transmission in colder climates and thought to be endemic throughout Europe at this time[26].

Compared with the early transect, 15 individuals recovered from the cemetery's middle and late phases exhibit greater variability in both genetic ancestry and *Plasmodium* species detected. Of the 13 male individuals, 11 have heterogeneous ancestry encountered across the Mediterranean, and 2 female individuals overlap the early phase cluster in PCA space (Fig. 4 and Supplementary Table 7). Interestingly, we identified *P. vivax*, *P. malariae* and/or *P. falciparum* in eight mid–late-phase male individuals, including three cases of multispecies *Plasmodium* infections, which are common today in geographic regions with more

than one endemic species[60]. To refine the possible source populations for these eight later-phase infected individuals, we performed further analyses using tools for ancestry spatial interpolation and modelling (Supplementary Note 10). As in the early phase, two *P. vivax*-infected individuals had ancestry similar to populations from central/northern Europe, consistent with a 'local' ancestry signature. For the remaining 'non-local' malaria cases, we narrowed down the possible sources to the southern Iberian peninsula ($n = 3$) and the Aegean ($n = 1$), and in two cases our modelling indicated mixed ancestry including both these former sources and Sardinia (Extended Data Fig. 10, Supplementary Table 8 and Supplementary Note 10). Remarkably, all individuals infected with *P. falciparum* and/or *P. malariae*, including the three individuals with multispecies *Plasmodium* infections, exhibited non-local ancestry. Low winter temperatures are thought to have restricted endemic *P. falciparum* foci north of the Alps[26], but these findings are consistent with the hypothesis that the mid–late-phase malaria-infected individuals from Mechelen may have been troops from the circum-Mediterranean region. More broadly, our results are consistent with the historical records regarding the army of Flanders, which in the sixteenth and seventeenth centuries CE recruited soldiers from northern Italy, Spain and other Mediterranean regions to fight in the Low Countries[61]. As well as providing compelling evidence regarding the mortuary context of these individuals, the host and pathogenic DNA retrieved raises important questions regarding the extent of local malaria outbreaks in this period. Notably, multiple anopheline vectors capable of transmitting *P. falciparum* and other malaria parasites persist in the Low Countries and other regions of Europe today[26,62]. Thus, although *P. falciparum*-infected individuals at Mechelen may represent isolated, recently imported cases, it is also possible that they fell victim to more-extensive local malaria outbreaks triggered by intense human mobilization in the socio-economic context of warfare.

Conclusions and implications

In this study, we demonstrate that malaria-parasite genome-wide mitochondrial and nuclear data can be reconstructed from human skeletal remains. Together with textual, osteological and archaeological evidence, these new biomolecular data provide an opportunity to reassess our understanding of the past distribution of malaria-parasite species. We show that *P. vivax* was endemic in Europe several thousand years before the earliest textual references, and the identification of *P. falciparum* in the Himalayan highlands and temperate Europe underscores the role of human mobility in carrying malaria to the peripheries of endemic zones. As well as species identification, we demonstrate that population genetic analysis of unsampled and eliminated parasite populations can provide critical insights into the sociocultural processes that helped to spread malaria around the globe. We find that eliminated European *P. vivax* resembles modern and ancient Latin American parasite populations, consistent with transmission from European colonizers to Indigenous peoples of the Americas in the contact period. We also found that American *P. falciparum* shows strong affinity to modern African lineages, implicating the trans-Atlantic slave trade in the spread of this parasite across the Atlantic.

Beyond these insights, the capacity to reconstruct ancient genomes from human malaria parasites raises new questions and opens multiple avenues for future research. The population history of European *P. falciparum* remains particularly enigmatic, with ancient strains showing relatedness to multiple extant modern lineages. Denser temporal and spatial sampling of European *P. falciparum* may help to elucidate whether these strains did indeed result from multiple admixture events or constitute a deeply diverged population without closely related extant lineages. More broadly, sampling of additional ancient and archival materials provides an opportunity to generate a more-comprehensive catalogue of *Plasmodium* diversity. Such efforts may be especially beneficial for regional populations in which successful elimination campaigns limit opportunities for sampling in public-health contexts. Similarly, although the near-fixation of the Duffy-negative allele limits *P. vivax* endemicity in sub-Saharan Africa today, ancient genome-wide data would provide an ideal opportunity to address debates regarding the geographic origins of this species. Despite preservation problems, our recovery of *P. vivax* DNA from ancient Egypt demonstrates that genotyping ancient *Plasmodium* strains from tropical and subtropical regions is theoretically possible. Finally, the ability to identify specific parasites in particular regions and time periods sets the stage for renewed study of the economic and human impact of malaria on past cultures. Integrating evidence from ancient DNA with historical records, osteological markers of anaemia and archaeological data could shed new light on historical debates, such as the possible role of malaria in the decline of the ancient Greek and/or Roman civilizations. Taken together, the capacity to reconstruct ancient genomes from *Plasmodium* spp. lays the groundwork for future studies on the origins, transmission, evolution and cultural impact of human malaria parasites.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-024-07546-2.

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