EVOLUTIONARY BIOLOGY

Genomic evidence for limited entomophagy in ancient Europeans

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To meet the rising food demands of our growing population, the Food and Agriculture Organization proposed edible insects as sustainable sources of animal protein. Although hundreds of million people already consume insects around the tropics, western societies remain averse to entomophagy. To trace whether ancient Europeans consumed insects, we here apply two complementary genomic approaches. Metagenomic screening on 745 ancient anatomically modern human dental calculus returned limited insect DNA traces, with read abundances well below those observed in Neanderthals, western chimpanzees, and gorillas. In addition, genes encoding stomach-expressed chitinases show two of the most significant signatures of latitudinal differentiation genome-wide. Clines are consistent with evolutionary benefits of entomophagy in tropical regions and with expression quantitative trait locus data supporting low chitin digestibility in present-day Europeans. Ancient genomes confirm that both clines already existed at the onset of agriculture and persisted despite massive migrations. Together, our findings support occasional and possibly incidental insect consumption in Europe over the past ~9000 years.

INTRODUCTION

Demographic models anticipate continued population growth for a few more decades (*1*). Feeding this expanding population will pose a substantial challenge because our planet is already overexploited, with ~35% of its terrestrial surface devoted to agricultural use (*2*). Transforming wild habitats into agricultural land could definitely accelerate biodiversity loss and exacerbate the ongoing sixth mass extinction (*3*, *4*). Traditional livestock not only demands vast amounts of land and water but also contributes substantially to greenhouse gas emissions as well as to pollution with nitrogen and phosphorus compounds (*5*, *6*). To bridge this looming protein gap while alleviating pressure on agrifood systems, the Food and Agriculture Organization (FAO) recommended using edible insects not only for feeding animals such as poultry, fish, and pets but also as novel ingredients for human food (*7*).

With 1611 edible species cataloged worldwide (*8*), hundreds of million people already eat insects regularly, especially around the tropics, where their biomass and diversity warrant stable harvesting (*9*–*12*). In many Indigenous communities, cultural memory and oral tradition preserve detailed knowledge of insect collection and preparation methods (*11*). Insects are valued not only as survival foods but also as celebrated components of regional cuisines, and their harvest provides a means of pest control (*13*). In contrast, cultural entomophagy is rare in modern Europe, although a few exceptions exist, such as casu marzu cheese and bee brood (*13*, *14*). Most Western consumers remain reluctant to eating insects (*15*), despite increased acceptance when insects are processed into flour to enrich bread, pasta, and cookies (*16*–*18*).

Historical chronicles can help trace the antiquity of this dietary practice. Although certain insect species were considered occasional delicacies by Greeks and Romans (*14*, *19*, *20*), insects were also responsible for crop losses that triggered devastating famines and pestilences through history, most notably caused by swarming locusts (*21*, *22*). With the spread of Christianity during Early Medieval times, insects became increasingly perceived as biblical plagues and categorized as censored foods in penitential books (*13*). Beyond historical chronicles, little is known about the role of insects in prehistoric diets (*14*, *23*, *24*), despite their potential importance during major shifts in human subsistence strategies. To travel further back in time and uncover insect consumption before written history, we apply here two complementary genomic approaches.

RESULTS

Metagenomic screening on ancient dental calculus

As calcified biomolecular archives of past dietary practices (*25*–*30*), ancient dental calculus may offer a unique substrate to search for genuine traces of insect DNA. After building a custom database of 10,761 insect mitogenomes (table S1), we used the Bayesian framework provided by HAYSTAC (*31*) to 1028 sequencing libraries from the dental calculus of 18 Neanderthals and 745 anatomically modern humans (AMHs), mainly ancient Europeans (tables S2 and S3). As a comparative benchmark, we additionally analyzed the dental calculus of 96 great apes with documented dietary habits, including insect consumption (table S2).

Insect DNA traces were found to be far more abundant in gorillas (Fig. 1), an herbivorous species. This likely reflects ingestion of foliage collected in large, indiscriminate handfuls (*32*, *33*), which can include species such as leaf-feeding and wood-boring beetles (*Monolepta signata*, *Psilothrix* sp., and *Callimerus inbasalis* in table S4; fig. S1).

Western chimpanzees from Nimba (Liberia) inhabit forest-edge, seasonal habitats where dense colonies of social insects, such as termites, help buffer protein and micronutrient shortfalls (*34*). Consistently, the dental plaque of this subspecies exhibited more insect DNA abundance than Eastern and Central chimpanzees. This included Blattodea from the family Termitidae, as well as grubs, caterpillars, and ants (Fig. 1 and figs. S1 to S4). In contrast, Eastern and Central chimpanzees occupy stable rainforests, with reliable leaf and especially fruit availability, making insectivory largely unnecessary, accounting for only <4% of their feeding budget (*35*). Insect consumption is nearly absent in chimpanzees from the Kibale National Park (Uganda; Eastern Africa) (*34*, *36*–*38*), from which seven of the samples analyzed here originate (table S2). Their low insect intake closely parallels the limited DNA abundance estimated for ancient

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Fig. 1. Insect orders identified in dental calculus from great apes and humans. AMH samples ($n = 745$) are grouped by geographic region, whereas Neanderthals ($n = 18$), gorillas ($n = 57$), and chimpanzees ($n = 39$) are grouped by subspecies. Groups are ordered horizontally according to descending mean insect DNA abundance. For each group, the order exhibiting the highest mean abundance is shown in yellow.

Eurasians, indicating that entomophagy was similarly rare among AMH from that region (Fig. 1).

Neanderthal dental calculus shows an insect DNA abundance comparable to Western (Nimba) chimpanzees, with a notable representation of Diptera (Fig. 1 and fig. S5). This may lend support to a recently proposed hypothesis, positing that regular consumption of animal corpses laced with fly maggots contributed to the elevated nitrogen isotope ratios ($\delta^{15}N$) observed in Neanderthals (*39*). The fact that Culicidae (mosquitoes) appear as the most abundant insect family within Diptera may align with Neanderthals preserving animal carcasses in ponds and swamps, where mosquitoes lay eggs. Our comparative benchmark therefore recapitulates known dietary patterns, supporting the value of dental calculus as an informative molecular archive of entomophagy.

Moving from high-order taxonomic assignments to confident species-level identifications requires additional quality filters and stringent validation criteria. These include ancient DNA authentication, species specificity, and high relative abundance. For authenticating ancient DNA, damage profiling represents the standard criterion. Unfortunately, this criterion is minimally informative in insects, due to their extremely low or absent DNA methylation (see Discussion) (*40*). Yet, reads mapping to insect mitogenomes are even shorter [mean length = 58.1 ± 14.4 base pairs (bp) after adaptor removal] than the remaining Illumina reads in the library (76.3 ± 13.9 bp), a pattern inconsistent with modern contamination but consistent with ancient DNA fragmentation (*41*).

Species specificity is supported by conditioning on reads with high mapping probability in HAYSTAC, by mapping against the few nuclear genomes available (table S5), and by MegaBLAST against the NCBI nonredundant database (table S6). All three benchmarks verified nearly exclusive alignment to the insect species identified. Within each sequencing library, reads of the identified insect species were at least as abundant as human reads. These insect reads did not cluster within the same mitochondrial region, indicating that their abundance was not inflated by (mis)alignment to evolutionarily constrained regions (evenness of coverage <2). Together, all these lines of evidence support the authenticity of the insect DNA signals, which were validated across two Neanderthal and eight AMH specimens, including 37 insect species corresponding to 41 hits (Fig. 2).

The Neanderthal Spy1 yielded molecular evidence of two insect species: *Psychoda alternata* and *Cricotopus trifascia*. The former is a moth fly that thrives in damp environments and whose larvae are capable of feeding on late-stage decaying matter such as animal carcasses (*42*–*44*). Although supported by two validated reads, of the 13.5 million tested, this represents the only species identified with certainty that could potentially link maggot consumption and elevated $\delta^{15}N$ values in Neanderthals.

Insect DNA in dental calculus showed otherwise a different profile, dominated by species associated with humid environments. For example, the calculus of PLV001_A [29,500 years before the present (yr B.P.); Czech Republic] (*36*) contained DNA from *Propsilocerus akamusi*, a nonbiting midge whose larvae develop in lake sediments and tolerate high levels of metal pollution and anoxia (*45, 46*). Although today restricted to East Asia, closely related species are widespread in European freshwater ecosystems (*47*), suggesting accidental ingestion while drinking. Similarly, OFN001_A (9200 yr B.P.; Germany) (*36*) and Abusir1519 (a 4000-year-old Egyptian mummy) (*48*) harbored traces of *Dorypteryx domestica* (supported by 580 reads, native to Africa) and *Liposcelis decolor* (*49*), which are typically associated with moist habitats such as grain storage facilities (*50*–*52*). These findings indicate that insects were likely consumed via contaminated foodstuffs rather than deliberate entomophagy, possibly even before the arrival of pottery and agriculture to Central Europe, as suggested by OFN001_A (supported by only seven reads, of the 105 million reads tested for this sample).

Egyptian mummies (e.g., Abusir) (*48, 53*) and century-old preserved skeletons from the Terry collection [Anonymous Research Participant (ARP) samples] (*54, 55*) carried DNA traces from a greater diversity of insect species than any other sample. Some of these species are necrophagous, such as the skin beetle *Dermestes lardarius*, likely reflecting postmortem colonization of decomposing human bodies (*56*–*58*). However, other species including *Periplaneta americana* and *Tribolium castaneum* are not necrophagous but secondary pests (*59, 60*), suggesting consumption of infested foodstuff, contamination during museum curation, and/or inaccuracies in sampling dental calculus.

Although overrepresented in mummies and preserved skeletons, postmortem contamination could also affect other samples. The Neanderthal GOY005_A (42,800 yr B.P.) (*36*) contained DNA from *Harmonia axyridis*, despite this lady beetle is native to Asia (*61*). This species was only introduced in Europe in the 1980s, intentionally (as an agent of biological control) and accidentally (with international trade of ornamental plants and crops) (*62, 63*). Now, it is a widespread pest in Northern Europe, including in Belgium (*64*), where the remains of GOY005_A were excavated (Goyet cave; Belgium) and rest.

Combined, our findings indicate that insect DNA traces were comparatively limited in AMH dental calculus and that most hits are best explained by postmortem contamination, accidental ingestion via carcasses, freshwater resources, or plant-derived foods such as stored grains, supporting that deliberate entomophagy was rare in Eurasia (Fig. 1).

Ancient selection signatures in stomach-expressed chitinase genes

It may still be argued that ancient metagenomics provide insufficient power to track insect consumption over time. Leveraging data from the 1000 Human Genome project (Phase III; Fig. 3A and table S7) (*65*), we complementarily scanned for signatures of clinal selection. We focused on the two GH18 family members encoding stomach-expressed enzymes, with catalytic chitinase activity. *Acidic Chitinase* (*CHIA*) is secreted into the stomach lumen, whereas *Chitobiase* (*CTBS*) is expressed in gastric endothelial cells, where it cleaves the chitobiose likely resulting from chitin breakdown, preventing its accumulation and lysosome storage disorders (*66, 67*). More specifically, we hypothesized that populations native to around the tropics underwent stronger selective pressures for enhanced chitin digestibility, given the present-day prevalence of insect consumption in these regions (*68*). This could result in pronounced latitudinal clines of genetic variation, possibly shaped and maintained over millennia.

After accounting for the underlying population history, we confirmed that *CHIA* and *CTBS* harbor mutations whose frequencies closely follow geographic latitude (i.e., measured distance to latitude 8°N; Fig. 3, B and C). These signals ranked among the most significant in the genome-wide single-nucleotide polymorphism (SNP) set, falling within the top 99.47 and 99.96% percentiles, respectively. Most of these outlier mutations clustered but were not necessarily adjacent, which indicates selection on distinct haplotype backgrounds (Fig. 3C). When scans were replicated at the haplotype level, based

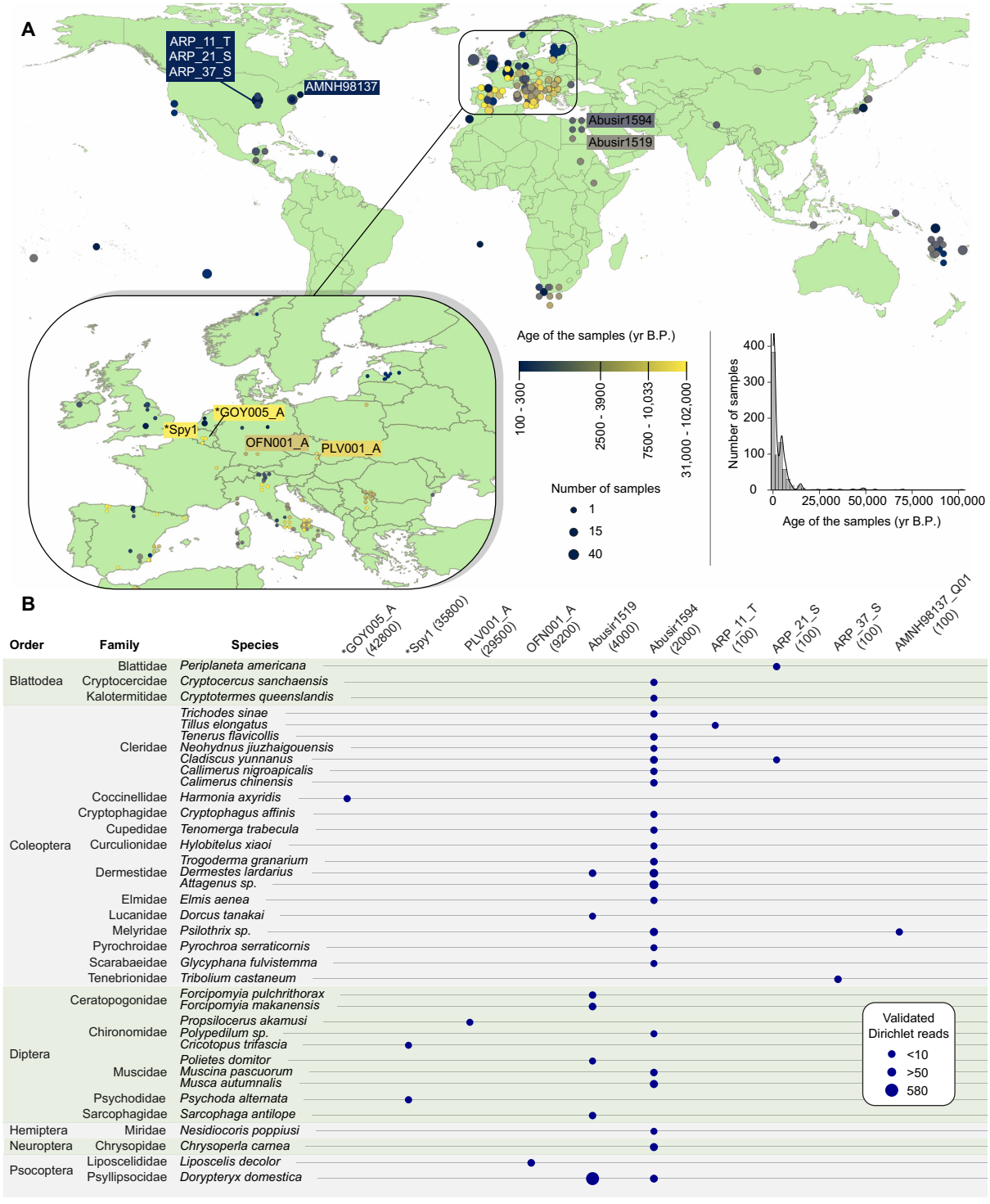

Fig. 2. Metagenomic screening on ancient dental calculus. (**A**) Geographic distribution of the 745 *H. sapiens* and 18 *H. neanderthalensis* (marked with "*" in their ID) used for metagenomic analyses, grouped by age and location. Point size is proportional to the number of samples, and color represents the sample age. The histogram in the bottom-right corner summarizes all sample ages (yr B.P.). (**B**) Samples whose dental calculus contain validated traces of insect DNA ($n = 10$), defined as the number of Dirichlet reads retrieved by HAYSTAC and later confirmed by MegaBLAST (validated Dirichlet reads). Insect species ($n = 37$) are taxonomically sorted, by order and family.

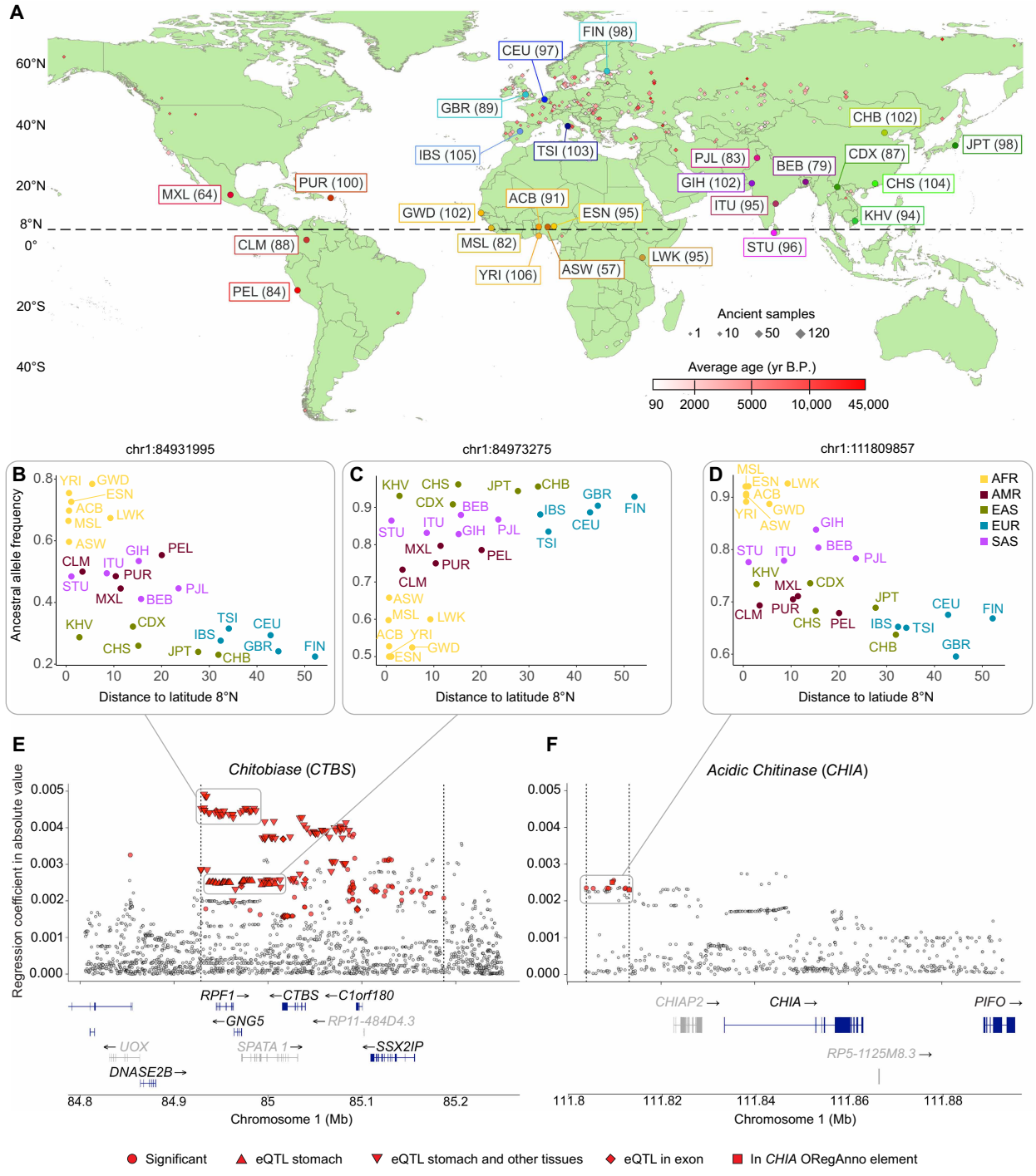

Fig. 3. Ancient selection in stomach-expressed chitinase genes. (**A**) Geographic distribution of the 26 modern populations (n = 2396) used for selection analyses, represented as circles with their three-letter population code and the number of samples retained after filtering. A few populations were (re)projected to better represent their native range (see Materials and Methods; table S7). Ancient samples (n = 1663) are shown as diamonds, and grouped by age and location to facilitate visualization. Point size is proportional to the number of samples in each ancient group, and color indicates the mean age of the corresponding samples. The horizontal dashed line delineates latitude 8°N, as a reference hotspot of insect consumption. (**B**) Relationship between the distance from each population to latitude 8°N and their respective ancestral allele frequencies, for an SNP showing significant association at position chr1:84931995 (*hg19* coordinates). (**C**) Same as (B) for chr1:84973275. (**D**) Same as (B) for chr1:11180957. (**E**) Distribution of significant SNPs (MAF > 0.10 and FDR-adjusted P value < 0.10) along the *CTBS* region. Vertical dashed lines delimit genomic regions considered to evolve under selection, according to the clustering of significant variants (colored in red). These regions were later used to define haplogroups. Point shapes denote overlap with known regulatory elements. Gene models (in blue) and pseudogenes (gray) are shown in the bottom. (**F**) Same as (E) for the *CHIA* region.

on the predefined boundaries delineated in Fig. 3C, patterns of latitudinal differentiation became highly significant for two *CHIA* and two *CTBS* haplogroups (adjusted *P* value < 0.025; Fig. 4A). Genome-wide, few other haplogroups showed comparable geographic trends (Fig. 4B). In addition, no other bioclimatic variable (*69*) predicted *CHIA* and *CTBS* haplogroup frequencies significantly better than distance to the tropics (table S8), supporting the latter metric as a more robust predictor of insect biomass and diversity (*68*).

In *CHIA*, the selected region lies upstream of the coding sequence and encompasses regulatory elements known to specifically control its transcription (e.g., OREG1508227 and OREG1858975) (*70*). Because *CHIA* expression is markedly higher in the stomach than in other organs, latitudinal selection can be tentatively linked to enhanced chitin digestibility. In contrast, *CTBS* presents a more ubiquitous transcriptional profile, raising the possibility that its differentiation reflects pressures unrelated to entomophagy, such as defense against chitin-containing microbes and fungi. Using expression quantitative trait locus (eQTL) data; however, we corroborated that alleles favored in tropical populations enhance *CTBS* expression in the stomach (fig. S6), with less significant impacts in other organs (table S9). Furthermore, other five chitinase GH18 family genes expressed outside the stomach show no latitudinal differentiation (*CHIT1*, *CHI3L1*, *CHI3L2*, *CHID1*, and *OVGP1*), including *CHIT1* with catalytic activity and expressed in immune cells. Together, these findings indicate that entomophagy, rather than resistance to biotic stressors, drove the observed geographic clines, representing one of the most significant signatures of latitudinal differentiation across the human genome and thus one of the most fitness-enhancing behaviors in tropical latitudes.

To characterize the temporal context in which these latitudinal gradients took shape, we leveraged 1663 ancient genomes released by Allentoft *et al.* (*71*), all imputed and phased. This genome dataset includes samples from Africa and America, but it is mainly comprised of ancient Eurasians. Within Eurasia, the frequencies of the two *CHIA* and two *CTBS* haplogroups showing latitudinal differentiation remained nearly steady over the past 9000 years (Fig. 5 and tables S10 and S11). This indicates that both clines were already present and have been maintained at least since the advent of agriculture

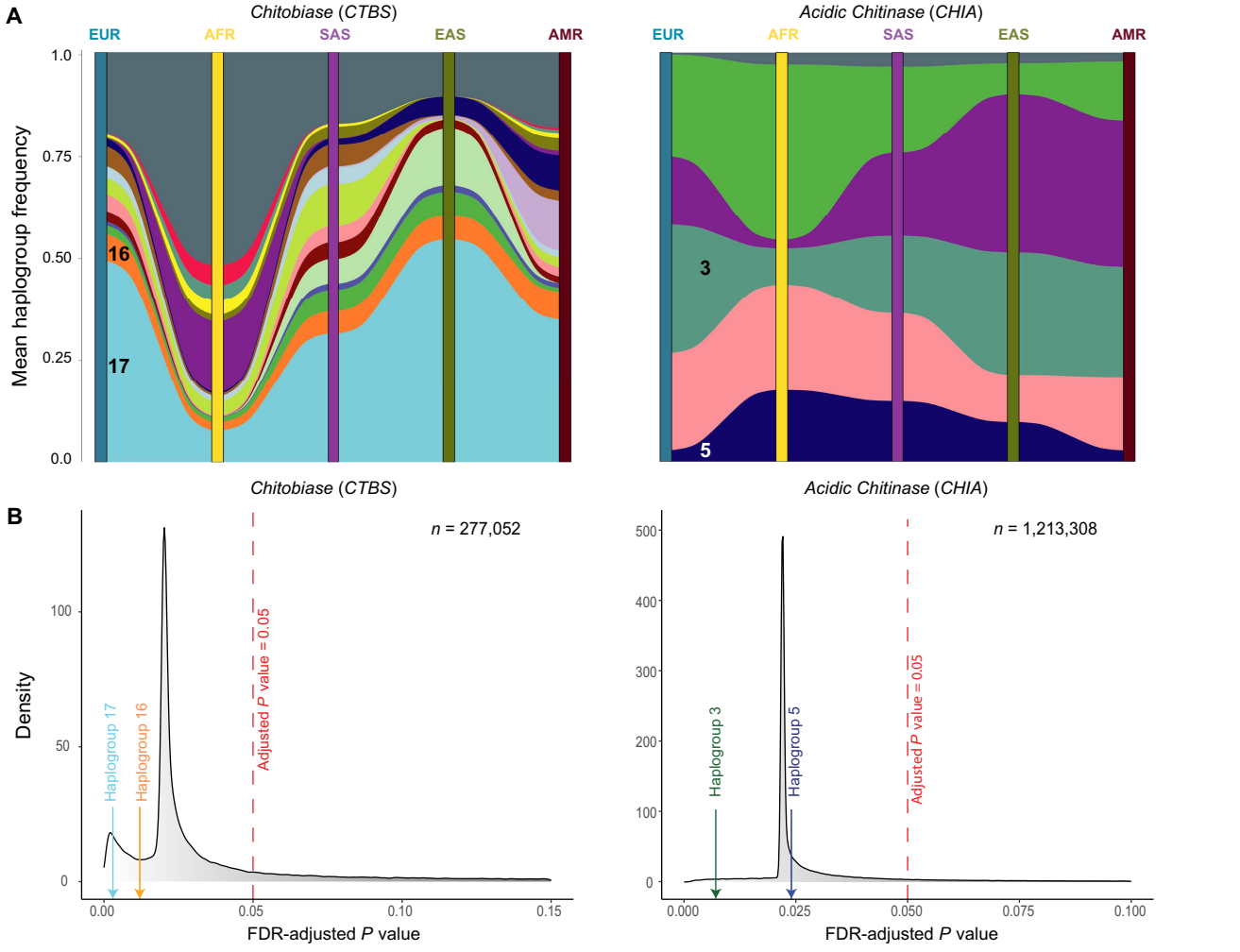

Fig. 4. Haplogroup composition in the *CTBS* and *CHIA* selected regions. (**A**) Shift in haplogroup frequencies across present-day superpopulations, for both *CTBS* (0.111500 cM) and *CHIA* (0.001434 cM). (**B**) Regression significance for *n* = 277,052 and *n* = 1,213,308 haplogroups distributed along the genome and matching the genetic length of the *CTBS* and *CHIA* regions under study (MAF > 0.01). Two *CTBS* (16 and 17) and two *CHIA* (3 and 5) haplogroups show greater statistical significance (FDR-adjusted *P* value < 0.025) than the vast majority of haplogroups.

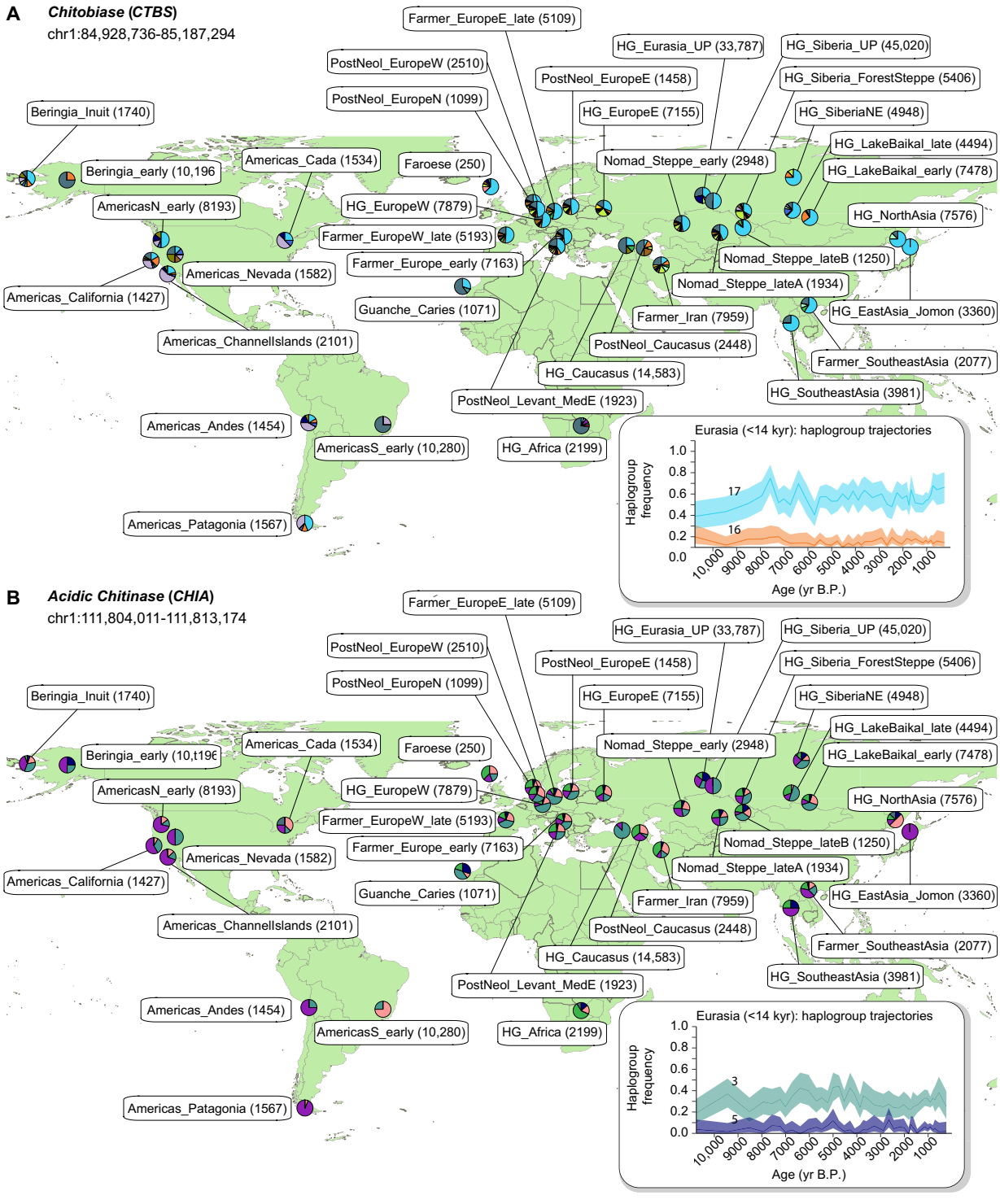

Fig. 5. Geographical and temporal distribution of *CTBS* and *CHIA* haplogroups. (**A**) *CTBS* haplogroup composition for all the ancient population clusters (*cluster_group* category) defined by Allentoft *et al.* (*71*). The inset shows Eurasian haplogroup frequencies in temporal bins of at least 100 years, for *CTBS* haplogroups 17 and 16 ($N = 1537$). For each bin, the mean is shown, with confidence intervals calculated via the exact two-sided 95% Clopper-Pearson method for binomial proportions. (**B**) Same as (A) for *CHIA*.

(*72*). According to all 130 eQTL SNPs in this dataset, ancient Eurasians systematically carried *CTBS* alleles that reduced gene expression in the stomach (fig. S6). Notably, these 130 *CTBS* alleles were found even at greater frequencies in relict forager communities such as the Jomon (Fig. 5), suggesting that the Eurasian predisposition for limited chitin expression existed before the spread of agriculture. In contrast to Eurasian AMHs, the two Neanderthals and the Denisova samples included in this dataset carried alleles associated with enhanced chitin digestibility, in line with the greater insect DNA abundance found in Neanderthal dental calculus (Fig. 1).

DISCUSSION

Given the growing industrial and academic interest in insects as food and feed (*7*), our study investigates whether aversion to entomophagy in Western societies dates to Early Medieval times or insect consumption was already anecdotal before. We draw on two independent lines of genomic evidence, both robust and converging on the conclusion that insect consumption was rare and likely unintentional in prehistoric Europe, at least since the Neolithic Revolution. This does not exclude intentional, sporadic insect consumption during (pre)history, especially during famines and epochs of nutritional emergency.

Our large-scale metagenomic screening involved a recently developed Bayesian method designed to minimize false positives (*31*), combined with strict quality filters to exclude low-complexity reads. Damage profiling, a standard authentication criterion for ancient DNA, is effectively uninformative for insects. This approach relies on the deamination of cytosines, either to thymines or to uracils, depending on whether the cytosine was originally methylated or not. Although uracils can be removed by Uracil-Specific Excision Reagent (USER) treatment, deamination in methylated cytosines can still inflate the C > T substitution rate (and complementary G > A) toward read termini, yielding a profile characteristic of ancient DNA molecules in vertebrates. In insects, however, CpG methylation levels are extremely low (~2% versus ~75% in mammals), and *Diptera* have entirely lost their enzymatic machinery for DNA methylation (*40*). As a result, USER-treated insect libraries rarely show terminal substitution patterns diagnostic of ancient molecules. Nonetheless, our reads were substantially shorter than expected for Illumina sequencing platforms, in line with fragmentation of ancient DNA molecules. Furthermore, insect reads were further validated to ensure (nearly) species-specific hits and were at least as abundant as the human (host) mitochondrial DNA in the same sequencing library. Together, these criteria support the legitimacy of our metagenomic results.

However, the presence of genuine ancient insect DNA in shotgun libraries does not necessarily imply entomophagy. Postmortem contamination is evident in mummies and other embalmed skeletons. They include necrophagous species and recognized pests, a contamination likely reflecting skin-associated material adhering to dental calculus during sampling, which aligns with the enrichment of skin-associated microbes in their sequencing data (*48, 55*). A similar contamination pattern applies to G0034, a historical gorilla stored in the Royal Museum for Central Africa (Belgium), whose dental plaque contained necrophagous *Diptera* species uncommon in Africa.

More intriguing is the infiltration of environmental DNA into clean skeletons, with no soft tissue remains. This is the case for GOY005_A (42,800 years old; Belgium), a Neanderthal whose dental calculus is contaminated with a pest species introduced in Europe less than 50 years ago. These findings call for systematic evaluation of sampling protocols and caution against bluntly assuming that dental calculus remains sealed from exogenous genetic material. Entomological criteria alone are not always diagnostic, meaning that postmortem adulteration may go undetected for other samples. The insect metagenomic profile reported for AMH, dominated by species linked to humid environments, may appear suspicious in this regard. It echoes patterns observed in bacterial metagenomic screenings of dental calculus, which recovered taxa commonly found in water columns and likely associated with postmortem contamination (*73*).

Even assuming that this infiltration of environmental DNA in humid environments could represent true instances of entomophagy, insect DNA was nearly absent in AMH dental calculus, in contrast to herbivorous gorillas and western chimpanzees (Fig. 1). Our comparative benchmark places AMH insect consumption at levels comparable to rainforest chimpanzees, well known for their limited entomophagy, of <4% feeding budget (*35*). This time investment likely translates into lower fractions in terms of biomass consumed because ant fishing and termite fishing are socially embedded, time-consuming activities for chimpanzees, which yield limited caloric returns.

Insects provide a cost-effective caloric return only when harvested in large quantities (*74*). Such insect abundance correlates more strongly with the proximity to tropical regions than with any other bioclimatic variable (table S8) or socioeconomic factor, including gross domestic product or arable land proportion per country (*68*). Consistent with this ecological pattern, the clines delineated by the two *CHIA* and two *CTBS* haplogroups stand out among the most significant signatures of latitudinal differentiation genome-wide. According to eQTL expression data, their underlying selective pressures likely involved chitin digestibility, rather than resistance against chitin-containing pathogens. Furthermore, our findings reveal that relict Eurasian foragers carried alleles associated with low chitin digestibility, suggesting these latitudinal clines were already established before the spread of agriculture. Combined, these observations align with the view that insect abundance near the tropics drove the geographic distribution of entomophagy and ultimately promoted enhanced chitin digestibility in tropical populations, a latitudinal pattern that has been maintained despite potential processes of Neolithization, Westernization, and syncretism.

The fact that insect abundance emerges as the ultimate driver of entomophagy supports the global mission of an emerging agricultural sector, aiming at mass-rearing a resource that has long been crucial for human fitness around the tropics, especially for the high nutritional needs of children (*7*). In populations carrying European ancestry, the low chitin digestibility may have compromised insect consumption in prehistoric times and contributed to strengthen aversion beyond cultural and religious motivations. Nowadays, insect processing and chitin separation (chitin is also a valuable industrial by-product) (*75*–*78*) could overcome such a limitation in European and descendant populations, thereby consolidating the agricultural sector of insects for food and feed, also in Western societies.

MATERIALS AND METHODS

Ancient dental calculus metagenomics

We compiled all available human dental calculus sequencing libraries from the AncientMetagenomeDir (AMDir) database (https://spaam-community.org/amdirt/, version v24.09.0) (*79*), which were further complemented with the Velsko *et al.* 2024 dataset (*80*). In total, we screened 1028 libraries from 745 samples of *Homo sapiens* and 18 *H. neanderthalensis*.

All sequencing libraries were preprocessed with BBMap (https://sourceforge.net/projects/bbmap/) to eliminate low-complexity reads (flags "minlen=30 filterpolyg=40 qin=33 mingc=0.2 maxgc=0.8 minbasefrequency=0.05 entropy=0.5"). Metagenomics analyses were then performed with HAYSTAC (*31*) (https://github.com/antonisdim/haystac, v0.4.12), against a reference database of 10,761 insect mitogenomes (table S1). These were retrieved from the NCBI Nucleotide database (30 October 2024), using the following query: "Insecta"[Organism] AND "mitochondrion"[All Fields] AND ("5000"[SLEN] : "50000"[SLEN]) NOT "UNVERIFIED"[All Fields] NOT "whole genome"[All Fields]). We also included the *H. sapiens* mitogenome [revised Cambridge Reference Sequence (rCRS)] to assess the human DNA content in each sequencing library. The final HAYSTAC database was built using the command database with the --accessions-file and --resolve-accessions options. This reference database was then formatted using "haystac database build –mtDNA," and the analyses were conducted with "haystac analyse --mode abundances --min-prob 0.50."

Posterior abundance estimates (Fig. 1) were then carefully examined to validate taxa as true positives, based on three stringent criteria. First, human mitochondrial DNA was identified in 606 libraries, showing the evenness of coverage centered around one, indicating uniform depth of coverage, except for five outliers yielding values higher than five. Hits against the human mitogenome were used as a baseline cutoff, meaning that insect taxa were considered true positives if their abundance was comparable to that of human reads. Insect taxa with the evenness of coverage values higher than two, or with a number of Dirichlet reads lower than two, were also excluded. Second, we assessed whether read lengths followed the expected patterns of DNA fragmentation, commonly found in ancient DNA (*41*). Whereas the mean DNA fragment length was 76.3 bp (± 13.9 bp) per library, reads underlying insect hits was 58.1 ± 14.4 bp, both compatible with aDNA fragmentation.

Reads that successfully mapped to a taxon were extracted and used as input for blastn megablast (web version, default parameters), which almost systematically confirmed the specificity of the signal (tables S4 and S6). In addition, if a reference genome was available for the identified insects, or for a species from the same genus, the Paleomix (*81*) workflow (v1.3.8) was used to map the raw sequencing reads to the available reference genomes (table S5). Reads were adapter trimmed with AdapterRemoval (minimum length = 25 bp, Ns and low-quality bases trimmed, including collapsing of overlapping paired-end reads) and aligned with Bowtie2 using end-to-end and sensitive settings. Only reads with a mapping quality of ≥25 were retained. Polymerase chain reaction (PCR) duplicates were removed, and coverage and depth statistics were generated for each library. This returned thousands of read alignments against the reference assembly, further validating species identification (table S5).

As a comparative control, we also analyzed the dental calculus of historical great apes, including 57 chimpanzees and 39 gorillas from diverse wild populations (*30, 36, 82, 83*) (table S2). This analysis was based on exactly the same parameters and insect mitogenome database as for humans, with the only difference being the inclusion of each species mitogenome instead of the human reference.

Quality filtering the 1000 Human Genomes

Variant Call Format (VCF) files from the 1000 Genomes Project (https://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/; Phase 3) (*65*) were concatenated with bcftools v1.19 (*84*), conditioning on autosomal biallelic SNPs with minor allele frequency (MAF) > 1%. This yield 12,051,488 SNPs segregating among $n = 2504$ individuals from $n = 26$ populations.

Pairwise genetic distances were calculated with PLINK (*85*) v1.90b7, using "--distance square 1-ibs flat-missing." Individuals showing atypical intrapopulation diversity, identified via the interquartile range criterion for outlier detection, were considered recent immigrants and excluded from further analyses as they are unlikely to represent the genetic background of their assigned population. Figure 3 and table S7 show the individuals retained after filtering.

Using these filtered populations, we next estimated chromosome-specific pairwise covariance matrices (Σ), with AdmixtureBayes (*86*). These matrices capture the genomic affinities between all individual pairs, inherently accounting for population structure. Pairwise covariances were calculated relative to the ancestral allele, defined by the ancestral reconstruction provided by the 1000 Human Genome Project (i.e., the AA field of the INFO column). This resulted in a final dataset with 12,032,264 variants across $n = 2396$ individuals (table S7).

We revisited the geographic coordinates of seven populations (of 26) included in the 1000 Human Genomes Project to better reflect their native ranges. We reassigned CEU (Utah Residents with northwestern European ancestry) coordinates to Brussels (50.85°N, 4.35°E). For GIH (Gujarat Indians in Houston), we assumed the coordinates of Gujarat (15.22°N, 72.66°E); for ITU (Indian Telugu in the UK), we assumed the coordinates of Andhra Pradesh (8.51°N, 80.52°E); and for STU (Sri Lankan Tamil in the UK), we assumed the coordinates of Sri Lanka (1.10°N, 79.92°E). For ACB and ASW, we assigned the coordinates of YRI (7.40°N, 3.92°E), and for MXL, we assigned the coordinates of Mexico City (19.42°N, 99.14°W).

Generalized least square models on SNPs

To quantify whether population allele frequencies (dependent variable *AFpop*) followed a pattern of latitudinal differentiation, indicative of selection, we implemented a total 12,032,264 generalized least square (GLS) regression models, in the form

$$AFpop \sim \beta 0 + \beta 1 \cdot Distance\_latitude\_8 + \varepsilon, \quad \varepsilon \sim \mathcal{N}(0, \Sigma)$$

In these models, population allele frequencies (*AFpop*) were regressed on *Distance_latitude_8*, defined as the latitudinal distance from the geographic origin of the population to latitude 8°N, which approximates the hotspot for maximum insect consumption (*9*). Population allele frequencies were computed according to the ancestral allele.

To control for demographic history, regression models were run separately per chromosome, incorporating the chromosome-specific covariance matrix (Σ). Because AdmixtureBayes corrects genetic distances to account for multiple mutations per site, covariance matrices were rescaled back between 0 and 1 using the function $covariance\_transformed = e^{-covariance}$. After this, the lower triangular matrix was passed to the corSymm() function, defining a fixed symmetric structure that was then supplied to the gls() function of the R package nlme (v3.1.152) (*87*). Alleles correlating with geographic latitude more than expected under this neutral demographic model were considered candidates for selection.

The resulting *P* values were corrected for multiple testing using the false discovery rate (FDR) method, as implemented in the p.adjust function (stats v4.0.4) (*88*). In Fig. 3C, an SNP was considered significant (i.e., colored in red) if its adjusted *P* value is below

0.10 and its MAF is higher than 10%. These criteria account for instances of soft sweeps and avoid the inclusion of almost fixed variants, respectively.

We specifically inspected for signatures of latitudinal selection in the two chitinase genes of interest, namely, *CTBS* (hg19; chr1:85,020,682–85,040,098) and *CHIA* (hg19; chr1:111,833,473–111,863,185). In addition, we also explored 200 kb upstream and downstream of their given gene coordinates to identify SNPs following significant latitudinal clines in both coding and regulatory regions (Fig. 3C). Regulatory annotations were queried in the GTEx portal (https://gtexportal.org/home/) (*67*) and in the ORegAnno database (*70*), still available through the University of California Santa Cruz genome browser (https://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/).

Clustering of haplogroups in modern and ancient data

Our previous GLS analyses revealed clusters of SNPs whose frequency across populations correlated with geographic latitude, in both *CHIA* [chr1:111804011–111813174, 9163 bp, 0.001434 centimorgan (cM), and 51 SNPs] and *CTBS* (chr1:84928736–85187294, 258,558 bp, 0.111500 cM, and 979 SNPs) (Fig. 3, B and C).

To identify whether such a density of significant SNPs reflects selection on haplotypes, we applied the hierarchical density-based spatial clustering of applications with noise (HDBSCAN) method, as implemented in the R function hdbscan of the library dbscan v1.2.2 (*89*). The *minPts* argument, which controls the minimum number of haplotypes necessary to define a cluster, was tuned to 48 (roughly 1% of the 4792 haplotypes of the modern dataset). This produced five clusters (haplogroups) for *CHIA* and 17 clusters for *CTBS*, respectively (Fig. 4). Cluster 0 collects haplotypes that remain unassigned to a cluster.

Haplotypes were also extracted from the Allentoft *et al.* 2024 dataset (*71*), which includes 1663 phased ancient genomes. We used the function predict of dbscan (*89*) to assign each ancient haplotype to haplogroups predefined based on modern samples. SNPs not present in the modern and ancient haplotypes were excluded from this analysis.

GLS models with haplogroups

To compare *CHIA* and *CTBS* haplogroups to genome-wide distributions, we partitioned each chromosome in nonoverlapping windows of equivalent genetic length (0.001434 and 0.111500 cM, respectively). Genetic distances were derived from publicly available genetic maps (COMBINED_LD; https://www.well.ox.ac.uk/~anjali/AAmap/maps_b37.tar.gz) (*90*).

For each SNP absent from the genetic map (e.g., at position p), we located its flanking genetic markers (positions $p1$ and $p2$) and linearly interpolated its expected recombination rate according to

$$cM(p) = cM1 + \frac{p - bp_1}{bp_2 - bp_1} x(cM2 - cM1)$$

SNPs falling outside the genetic map boundaries were thus excluded. We also discarded genomic windows deviating by more than 15% from the targeted genetic length. Within each genomic window, haplogroups were automatically defined using the HDBSCAN workflow described above.

This procedure yielded two haplogroup datasets: one for *CHIA* and another for *CTBS*. These were used as independent null models. We replicated the GLS analyses based on haplogroup frequencies, but only if their MAF was superior to 1% (and below 99%). The only other difference is that covariance matrices, based on haplogroups, were computed using in-house scripts, as

$$\Sigma_{ij} = \frac{1}{H} \sum_{h=1}^{H} (p_{h,i} - o_h)(p_{h,j} - o_h)$$

where $p_{h,I}$ is the frequency of haplogroup h in population I, o_h is the frequency in the ancestral sequence reconstruction, and H is the total number of haplogroups across all windows (1,213,308 haplogroups for *CHIA* and 277,052 for the *CTBS*). The covariance matrices were provided to the gls() function without additional transformations. We adjusted P values via the FDR method and checked which haplogroups of the target regions had a value lower than 0.05 and a frequency higher than 5%.

To assess whether other covariates could explain haplogroup latitudinal differentiation, we fetched 19 bioclimatic indicators from WorldClim that collectively characterize pluviometry and temperature (*69*). Equivalent regression models were conducted, returning no better fit than *Distance_latitude_8* (table S8). This indicates that *Distance_latitude_8* represents an excellent proxy for insect biomass and diversity around the tropics (*68*).

Haplogroup temporal trajectories

We selected individuals from Eurasia no older than 14 thousand years (kyr) ($n = 1537$) and grouped them by age (*age_average* variable) into contiguous temporal windows of 100 years, which were extended forward in time until containing at least 25 individuals. Mean haplogroup frequencies were recorded for each time bin, and exact two-sided 95% Clopper-Pearson confidence intervals were computed with binom.test (stats v4.4.1) (*88*) (tables S10 and S11). To facilitate visualization, only haplogroups showing a significant geographic cline, as defined by GLS models, were displayed (Fig. 5).

Statistical analyses

All statistical analyses were performed using R (www.r-project.org).

Supplementary Materials

The PDF file includes:

Figs. S1 to S6

Legends for tables S1 to S11

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S11

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