Pathogen prospecting of museums: reconstructing malaria epidemiology

Nelder, M.P.; Schats, R.; Poinar H.N.; Cooke A.; Brickley M.B.

PNAS PERSPECTIVE

Pathogen prospecting of museums: Reconstructing malaria epidemiology

Mark P. Nelder[a,1], Rachel Schats[b], Hendrik N. Poinar[c,d,e], Amanda Cooke[c], and Megan B. Brickley[c]

Edited by Jane Buikstra, Arizona State University, Tempe, AZ; received July 28, 2023; accepted February 6, 2024

Malaria is a disease of global significance. Ongoing changes to the earth's climate, antimalarial resistance, insecticide resistance, and socioeconomic decline test the resilience of malaria prevention programs. Museum insect specimens present an untapped resource for studying vector-borne pathogens, spurring the question: Do historical mosquito collections contain *Plasmodium* DNA, and, if so, can museum specimens be used to reconstruct the historical epidemiology of malaria? In this Perspective, we explore molecular techniques practical to pathogen prospecting, which, more broadly, we define as the science of screening entomological museum specimens for human, animal, or plant pathogens. Historical DNA and pathogen prospecting provide a means of describing the coevolution of human, vector, and parasite, informing the development of insecticides, diagnostics, therapeutics, and vaccines.

evolution | genomics | museomics | vector-borne disease | paleopathology

Malaria is just behind tuberculosis and measles as the world's deadliest infectious disease, making malaria surveillance, prevention, diagnosis, and treatment global health priorities. Malaria is a mosquito-borne disease caused by the apicomplexan parasites *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi* (*SI Appendix*, Table S1). *Plasmodium* are transmitted to humans through bites of *Anopheles* mosquitoes, predominantly in tropical and subtropical regions. Macaques are the natural hosts of the zoonotic *P. knowlesi*, while the remaining *Plasmodium* are obligate parasites of humans. The World Health Organization (WHO) detailed 249 million cases and 608,000 deaths globally in 2022, with an inordinate proportion of morbidity (94%) and mortality (95%) foisted upon Africa (1). Children under the age of five are especially vulnerable to severe outcomes of infection such as acidosis, anemia, convulsions, impaired consciousness, and respiratory distress. A further sobering reality of the disease is that children less than 5 y old account for 76% of global malaria deaths.

Before 1900, malaria and its bygone epithets of aestivo-autumnal fever, ague (paroxysms of chills, fevers, and sweats), marsh fever, Roman fever, swamp fever, and vernal fever occupied a wide swath of temperate Asia, Europe, and North America (Fig. 1 and *SI Appendix*, Fig. S1) (2). By 1975, for the most part, malaria was eliminated from temperate regions through improved standards of living that limited mosquito biting (e.g., window screens, air conditioning); improved healthcare and public health infrastructure; use of the insecticides DDT and Paris green (copper acetoarsenite); management of standing water habitats through swamp drainage or oiling; increased agriculture leading to *Anopheles* preferentially blood-feeding on livestock over humans; and early and improved treatment of parasitemia with quinine, chloroquine, and mepacrine (3, 4). Owing to perennial *Plasmodium* transmission and heat, the tropical cradles of malaria did not experience the elimination successes of temperate regions. Nations currently malaria-free or with a reduced burden of malaria are at risk of disease resurgence. In 2023, local transmission of *P. vivax* (AR, FL, and TX) and *P. falciparum* (Maryland) in the USA highlights the ever-present threat of malaria wherever competent vectors are present (e.g., *P. vivax*-positive *Anopheles crucians* in Florida) (5, 6).

As one of the deadliest and most burdensome diseases, malaria lends itself to intense study. Research funding like the Bill and Melinda Gates Foundation, and the realization of a malaria vaccine for children, offer hope for accelerated discovery in malaria epidemiology and host-vector-pathogen evolution (1, 8, 9). However, attention to the deep history perspective of the disease is also gaining momentum; with scientific discovery coming through rigorously executed studies (10–13). For instance, using next-generation sequencing (NGS) and a 1940s medical collection from Spain, Gelabert et al. (14) reconstructed a regionally eradicated *P. vivax* strain from blood smears on microscope slides. Phylogenetic analysis of the rebuilt *P. vivax* mitochondrial DNA (mtDNA) genome revealed a close relation to present-day strains circulating in South America. Using metagenomics and ancient DNA (aDNA, >200 y old) approaches such as capture-enrichment, Marciniak et al. (15) provide not only the "gold standard" for detecting human tissue-derived *Plasmodium* DNA, but the earliest evidence of *P. falciparum* infection dating to the first to

Author affiliations: [a]Enteric, Zoonotic and Vector-Borne Diseases, Health Protection, Public Health Ontario, Toronto, ON M5G 1M1, Canada; [b]Laboratory for Human Osteoarchaeology, Faculty of Archaeology, Leiden University, 2333 CC Leiden, The Netherlands; [c]Department of Anthropology, McMaster University, Hamilton, ON L8S 4L9, Canada; [d]Department of Biochemistry, McMaster University, Hamilton, ON L8S 4L9, Canada; and [e]McMaster Ancient DNA Centre, Department of Anthropology, McMaster University, Hamilton, ON L8S 4L9, Canada

Author contributions: M.P.N. and M.B.B. designed research; M.P.N., R.S., H.N.P., A.C., and M.B.B. performed research; and M.P.N., R.S., H.N.P., A.C., and M.B.B. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence may be addressed. Email: mark.nelder@oahpp.ca.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2310859121/-/DCSupplemental.

Published March 25, 2024.

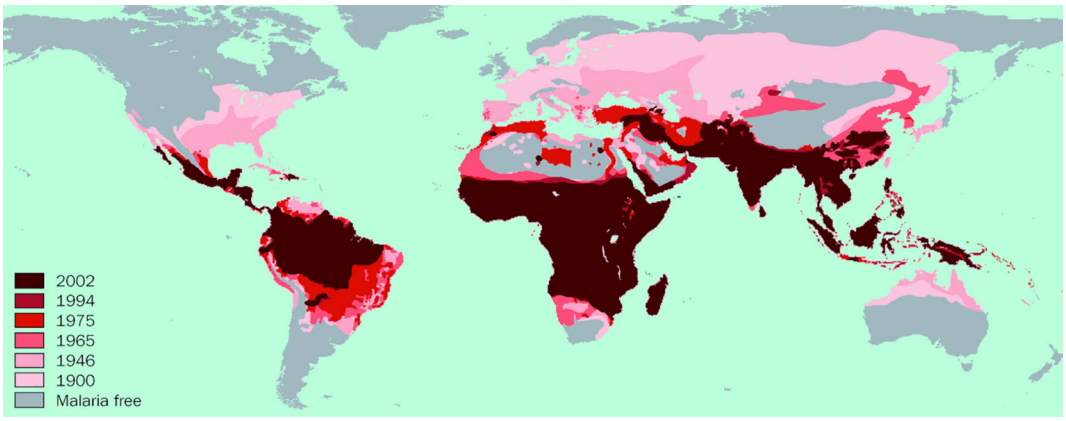

Fig. 1. Periodical snapshots of malaria's range (1900 to 2002). Used with permission. Image credit: adapted from ref. 7. CC BY 4.0.

second century CE Italy. The common thread among these studies is innovative linking of historical and contemporary disease.

In this Perspective, we examine an *Anopheles-Plasmodium* model of pathogen prospecting–defined broadly as the science of screening entomological museum specimens for human, animal, or plant pathogens. Enabulele et al. (16) have explored prospecting for zoonotic pathogens in museum-archived rodent tissues; but, to the best of our knowledge, pathogen prospecting for *Plasmodium* in museum *Anopheles* has not been studied. The intent of this Perspective is to prompt a discussion of pathogen prospecting and its application to malaria research, leading to the development of cross-disciplinary research and initial proof-of-concept studies. We focus on the detection of *Plasmodium* DNA in *Anopheles* held in museums, which will provide spatiotemporal data for reconstructing historical malaria epidemiology and evolution. There is a renewed enthusiasm for natural history collections and the opportunities they present, such that "*The ability to perform genomic sequencing on long-dead organisms is opening new frontiers in evolutionary research*" (17) (Shpak et al. 2023: 1).

A Historical Backdrop to Malaria's Epidemiology and Medico-Social Impact

Early Insights into Disease and Epidemiology. Nineteenth-century medical literature, though lacking laboratory diagnostics, meticulously describe what is unquestionably *Plasmodium* infection. Ringer (18) in 1859 England described a patient with tertian ague (likely caused by *P. vivax*), including temperature and pulse readings every 15 min. On days 1 and 3 of illness, chills (~37.7 °C) began at 10 AM and temperature rose to ~40.6 °C in 2 h, followed by slowly decreasing temperatures and sweats 1 h later. Intermittent chills, fever, and sweats were often the sole manifestations of mild disease, whereas, severe ague could manifest with depression, diarrhea, fatigue, headache, jaundice, loss of appetite, rapid breathing, and rapid heart rate (19). In 1890s Senegal, likely cases of falciparum malaria presented with remittent fever, malaise, and vomiting, followed by jaundice, delirium, pernicious anemia, and difficulty breathing (20).

Prior to the discovery that *Plasmodium* was transmitted to humans by anopheline mosquitoes (c. 1897 to 1898), malaria was thought to have originated as miasmas or poisonous gases (*mal'aria* = bad/corrupted air) from swamps (21). While scientific minds of the day were without the details of malaria, they nonetheless understood some of the basic principles of its epidemiology. For instance, MacCulloch (22) alluded to the habitats of immature mosquitoes being the source of malaria: "*The salt marshes of Normandy* [France], … *are notoriously productive of intermittents, to such a degree, that scarcely an inhabitant is exempt from them*" (MacCulloch 1827: 36). Furthermore, writers linked mosquitoes and malaria: "*The light curtains used as protections against mosquitoes in India have the same effect, and in the absence of either by simply covering the mouth and nostrils with a pocket handkerchief, a person may, in some measure at least, obtain protection*" (23) (Gordon 1874: 193); and, in reference to avoiding miasmas: "*Mosquitos too dislike an elevated breezy situation. Thus sleeping in upper rooms conduces to ventilation, to coolness, and to freedom from the plague of mosquitos*" (24) (Moore 1881: 301).

Malaria exacted a toll on the immunologically naive, like those living on malaria-free island nations or areas of unstable transmission. Such was the case for colonial Mauritius following the importation of *Anopheles gambiae* sensu lato (s.l.), *Anopheles funestus* s.l., *P. falciparum*, and *P. vivax* (25). During an 1867 outbreak in Mauritius with over 40,000 deaths, the American consul and naturalist Pike remarked, "*Fever was the only word on every lip, the only thought in every heart. Mourning and desolation were everywhere … May I never again witness the sad sight of those incessant funerals slowly winding along from morn till night*" (25) (Bruce-Chwatt and Bruce-Chwatt 1974: 1074–5). Another example is that of an expedition composed mostly of forced laborers from the malaria-free island of Rotuma (Fiji), tasked with harvesting sandalwood on the malaria-endemic island of Erromanga (Vanuatu) (26). Malaria took its toll on the 163 malaria-naive laborers that landed on Erromanga on January 22, 1830, as only 23 survived the return trip to Rotuma 2 mo later. Malaria also influenced prehistoric settlement patterns among the Southwest Pacific Islands, where the population density increased from west (Melanesia) to east (Polynesia), while malaria prevalence and transmission efficiency decreased (27, 28).

The Historical Medico-Social Impacts of Malaria. America typifies malaria's impact on humanity during Western colonialism. In Charleston, South Carolina from 1720 through

1754, malaria played a part in only 19% of the population reaching the age of 20 and rightfully earned the sobriquet "*great destroyer*" (29). Disease and mosquitoes were central to the abhorrent conditions endured by soldiers during the American Civil War (1861 to 1865), prompting a Union soldier to recount, "*We are more afraid of ague here than the enemy*" (2) (Miller 1997: 233). Besides increasing susceptibility to other diseases (e.g., cholera, typhoid), malaria had lasting effects on population health, leading to long-term sequelae and complications such as chronic anemia, cachexia, chronic kidney disease, enlarged spleen, liver failure, heart failure, immune disorders, and neurodegenerative deficits (30, 31). Yet, disease and death were not the sole outcomes of malaria, as they were, and still are, accompanied by economic hardship.

Poverty is part and parcel to malaria's morbidity and mortality, while the burden of malaria is reciprocally intrinsic to poverty. The personal financial burden of malaria includes costs associated with medical care, treatment, and lost wages due to illness or caring for ill family members (32, 33). Malarious countries are not only poorer than nonmalarious countries, but also experience stymied economic growth (e.g., decreased investment, labor force, agriculture, and tourism). In Bengal, India (late 19th century and early 20th century), areas with higher burdens of malaria endured economic declines brought upon by decreased agricultural activity and depopulation (34). Economic demise leads to the failure of national malaria prevention programs, especially when public health surveillance, vector surveillance, mosquito control, and treatment programs are underfunded.

Health and economic impacts are further exacerbated by malaria's role in inequity. Predominantly in those of African descent, malaria exerts strong selective pressure for increasing the frequency of red blood cell (RBC) alleles imparting varying levels of *Plasmodium* resistance; e.g., Duffy antigen receptor for chemokines (DARC) variant (FY*O allele), sickle-cell trait variant (HbAS allele), and β-thalassemia variant (β^+ allele) (35). A relatively small proportion of the variation seen in malaria resistance is explained by RBC polymorphisms, highlighting the complexity of resistance heritability and the potential role of epigenetics and polygenetics (35–37). The genetic basis for RBC structure and function, along with its importance, is becoming clearer, nonetheless, there is no consensus on the multiple mechanisms involved in malaria resistance (36, 38). The advantage of malaria resistance was tempered by colonists using these traits, in part, to justify continued chattel slavery. In colonial America during seasonal peaks of malaria, the Black or poor remained in warmer lowland areas along the coast with high transmission rates, while the White or wealthy retreated inland to the cooler highlands with less-intense *Anopheles* feeding (*SI Appendix*, Fig. S1) (29). Ancestry or social status often determined whether someone lived in a high or low transmission area, making malaria-influenced segregation commonplace throughout history; e.g., Sardinia (1,700 to 238 BCE), 17th to 19th century Ethiopia, and early 20th century Cameroon and Sierra Leone (39, 40). The medico-social impacts of malaria (i.e., poor health, poverty, and social inequality) are compounded by the factors governing the spread of vectors and parasites, altering the distribution and risk of disease.

Contributors to Vector/Pathogen Spread and Malaria Resurgence

Although advances have been made in malaria prevention, the disease still has a major global health impact. Now is the time to explore a historically based stratagem for understanding malaria evolution and epidemiology. Despite a great deal of uncertainty surrounding malaria's epidemiological and evolutionary past, factors contributing to increased historical disease risk are of particular interest when addressing challenges to present-day prevention efforts. Aspects of weather, climate, the anthropogenic landscape, and evolution frequently initiate a cluster of interlinked socioeconomic and sociocultural factors that often affect vulnerable groups the most.

Weather and Climate. Temperature, precipitation, and relative humidity are important to mosquito bionomics, including distribution, seasonality, survival, and abundance (Fig. 2) (41). Higher temperatures increase the potential of anopheline mosquitoes to transmit *Plasmodium* (vectorial capacity), partly through shortening the *Plasmodium* extrinsic incubation period–time to parasite development in *Anopheles* and transmissibility. In the WHO's World Malaria Report 2023, extreme monsoon rainfall and melting glaciers in Pakistan (2022) led to increased standing water and a fivefold increase in malaria incidence compared to 2021 (1). In the highlands of Ethiopia, a 1958 epidemic caused at least 3 million cases and 150,000 deaths among a population with little acquired immunity (42). Prior to and during the epidemic, Ethiopia faced increased precipitation, temperature, and relative humidity, all favorable conditions for *A. gambiae* and parasite proliferation; these conditions permitted for transmission of *P. falciparum* at high elevations (1,600 to 2,150 m).

Climate change makes previously unfavorable *Anopheles* habitats now favorable. In Africa (1898 to 2016), warming temperatures coincided with the range of *Anopheles* advancing south at 4.7 km/y and at an added elevation of 6.5 m/y (44). Warmer temperatures are projected (2070 to 2099) to expand malaria toward temperate regions and to lengthen the *Plasmodium* transmission season by 1.6 mo in the highlands of Africa, eastern Mediterranean, and Americas (41). In Europe, warmer springs, wetter summers, and wetter autumns are predictive of a northern range expansion of *Anopheles atroparvus* and *Anopheles messeae* (increased malaria risk) (45). In contrast, predicted drier conditions will shrink the ranges of several vectors in the eastern Mediterranean such as *Anopheles superpictus* (decreased malaria risk). Particularly relevant to Oceania, climate change increases vulnerability to not only malaria, but to rising sea levels, extreme weather, overcrowding, food insecurity, foodborne disease, and zoonotic disease (46). Predicting the impacts of climate change on malaria is complex and context dependent, and subject to syndemic factors (e.g., social, economic, political, and environmental) (47, 48).

Venezuela has faced a dramatic 365% increase in malaria cases from 2000 through 2015 (49). Venezuela's resurgence is due to shortage of antimalarials, underfunded prevention programs, ecological degradation from mining, and an ineffective healthcare system, all products of political unrest and economic collapse. In southern Venezuela from 1996

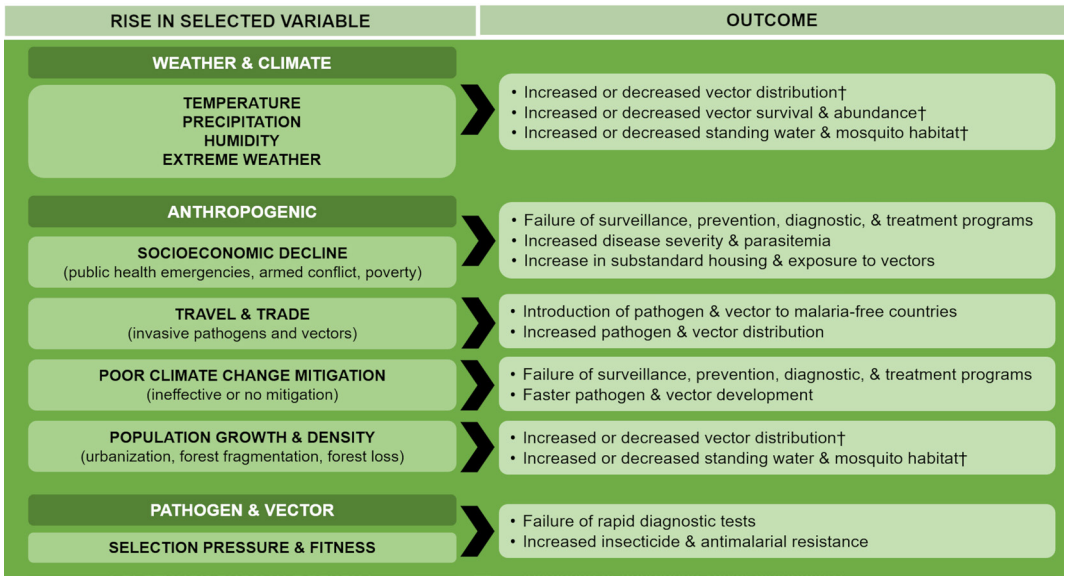

Fig. 2. Primary variables contributing to shifting vector/pathogen distributions and malaria risk. Variables and outcomes are presented as cause-and-effect examples; however, in reality, variables are highly interconnected and represent complex ecological processes with outcomes that are difficult to predict. †Context-dependent outcomes. For example, increased temperatures speed up mosquito development, blood-feeding rates, and parasite development (contributing to increased malaria risk). In contrast, the same higher temperatures can increase evapotranspiration, thereby decreasing soil moisture and standing water (contributing to decreased malaria risk) (43).

through 2016, increased temperatures and mining activity led to increased malaria incidence, highlighting risk at the intersection of climate- and human-driven influences (50). Similarly, following the large-scale deforestation and land use intensification in the Amazon basin after the 1960s, malaria incidence increased at pace, undoing much of the successful malaria control efforts before the 1960s (51).

Anthropogenic. Although tightly intertwined with weather and climate, human-driven variables also impact malaria risk, including globalization of vectors and pathogens, economic declines, inequitable access to healthcare, public health emergencies, and land use changes (Fig. 2) (1, 52, 53). Anthropogenically altered landscapes frequently provide additional suitable larval habitats for anopheline mosquitoes, including cisterns, culverts, ditches, dams, mining pits, rain gutters, and wells (54, 55). Traditionally, malaria has been a rural disease in Sub-Saharan Africa; however, *A. gambiae* s.l. are adapting to urban habitats and the newly introduced, urban-adapted *Anopheles stephensi* will place more people at risk (56, 57). Human-driven factors impacting *Plasmodium* transmission are complex and context-dependent with outcomes depending on vector ecology and malaria epidemiology; in other words, anthropogenic factors may increase or decrease malaria risk. During the post-deforestation period, agricultural development decreases mosquito biodiversity, leading to the proliferation of dominant vector species and malaria risk, while urbanization increases mosquito biodiversity, leading to decreases in efficient vectors and malaria risk (54, 58).

Pathogen and Vector. Evolution within *Plasmodium* and *Anopheles* contribute to increased malaria risks. For instance, deletions of *histidine-rich protein 2* (*pfhrp2*) genes in *P. falciparum* have emerged independently several times in Ethiopia–driven by a rapid diagnostic test-based test-and-treatment protocol–resulting in approximately 9.7% of infections going undiagnosed (59). Antimalarial drug resistance is an emerging global threat to managing malaria effectively, with resistance to artemisinin-based combination therapies (ACTs) emerging in Southeast Asia and resistance to aminoquinolines and antifolates in Africa (60). In Kayin State, Myanmar, armed conflict, the COVID-19 pandemic, and resistance to ACTs are testing the resilience of antimalaria programs in the region (1, 61). In 2020, there were ~8,000 malaria cases in Kayin State, increasing to ~32,000 in 2022 (62).

In a simplified three-species system of coevolution, humans, *Anopheles*, and *Plasmodium* each adapt at different times and rates along malaria's evolutionary timeline. The ability of humans to alter their environment, thereby changing selective pressure, means evolutionary change (i.e., bottleneck) can occur and spread quickly in vectors and pathogens (63). For instance, initial reports of chloroquine resistance in *P. falciparum* and DDT resistance in *Anopheles darlingi* emerged in ~20 y (64, 65). On the Cape Verde islands, admixture of Portuguese colonists with African enslaved individuals–who supported higher allele frequencies of the DARC variant–accelerated their adaptation to and protection against *P. vivax* infection in ~500 y or ~20 generations (66). Rapid evolution represents an additional threat to managing malaria, requiring novel, adaptation-resistant approaches to prevention.

Natural History Collections and Infectious Diseases

Historical records provide tantalizing clues to malaria's past, but we need new approaches to understand *Plasmodium* evolution. In addition to traditional taxonomic and biodiversity roles, natural history collections allow for infectious disease research that addresses aspects of evolution, ecology, medicine, and public health (Fig. 3) (67). Natural history

collections are conduits for understanding the trajectory of an infectious disease, from pathogen adaptation and emergence to epigenetic changes and shifting epidemiology. In addition, museum collections are a potential solution to the absence of spatiotemporal, species-specific records of *Plasmodium* transmission and malaria epidemiology.

Colonialism's role in usurping the natural history of malaria-burdened people should not be overlooked. The provenance of these collections in many cases rooted in legacies of colonialism, with specimens having been shipped to European museums as part of the cataloging of empire (69), and to museums founded in the colonies (70). Collections made during and since the colonial era contain critical historical information useful in the present, aiding those living in regions with increasing temperature anomalies and the highest risk of malaria.

Anthropological Collections. Paleomicrobiology (detecting human tissue-derived *Plasmodium* aDNA) is a progenitor of museomics [detecting hDNA (<200 y) of pathogens in human tissue samples], and museomics a progenitor of pathogen prospecting (71–73). Pathogen prospecting is the nexus for infectious disease research and collections conservation.

Paleopathology (the study of past disease in human and animal remains), similar to paleomicrobiology, is currently limited in what it can infer about prehistoric or historic malaria epidemiology. While several infectious diseases leave lesions on bones that have a high level of diagnostic certainty, malaria does not, despite the malarial parasite having serious consequences for human health (74). One means by which paleopathologists have attempted to access information on malaria is through studying evidence of anemia, as chronic anemia is a long-term sequela of malaria. However, the use of porotic lesions - which have multiple causes, particularly those of the orbital roof (cribra orbitalia) and cranial vault (porotic hyperostosis) as a proxy for anemia has confounded understanding of past anemia (75). Several studies have used sufficiently large samples and combined skeletal data with spatial analysis exploring geographic variables such as geology, topography, vector habitat, and anthropogenic landscape (76, 77). These studies demonstrate an important link between prevalence of porotic lesions and the landscape in regions of historically endemic malaria. However, it is not enough to say that the environment was conducive to malaria or the co-occurrence of vectors and human hosts. Future paleopathological research must take a spatial and temporal lens which is region specific, and which considers human behavior in relation to the landscape at the forefront of the research. To some extent, skeletal evidence of RBC disorders contributes to this spatiotemporal view. Both β-thalassemia and sickle cell disease leave some characteristic skeletal lesions. The former arising as a result of extensive marrow hyperplasia (expansion), such as rodent facies (changes to the facial bones) and rib-within-a-rib sign (observed in rib shafts) (78, 79); and the latter, bone infarcts (blocked blood flow leading to dead tissue) (80). While characteristic, there are no skeletal pathologies diagnostic of sickle cell disease. Despite the limitations, paleopathology is making advances in the understanding of malaria and human coevolution.

Paleomicrobiology looks to reconstitute ancient disease epidemiology and improve our understanding of present-day, emerging infectious diseases. Studies using aDNA protocols identified pathogen DNA in individuals infected with *Mycobacterium tuberculosis* (tuberculosis; first century CE Israel), *Mycobacterium leprae* (leprosy; first century CE Israel), and *Yersinia pestis* (Black Death; 14th century CE Europe); however, identification of human tissue-derived *Plasmodium* DNA is more complex (11, 81). Successful detection of *Plasmodium* DNA appears dependent on the level of parasite load at the time of death, species involved, and variables related to the preservation of human tissue-derived *Plasmodium* DNA (74). Schats (74) reviewed paleomicrobiology. Microbiological detection of human tissue-derived *Plasmodium* DNA has predominantly been PCR-based, targeting *18s rRNA*, *apical membrane antigen 1* (*ama1*), or *P. falciparum chloroquine resistance transporter* (*Pfcrt*). Most recent studies make use of newly developed capture-enrichment

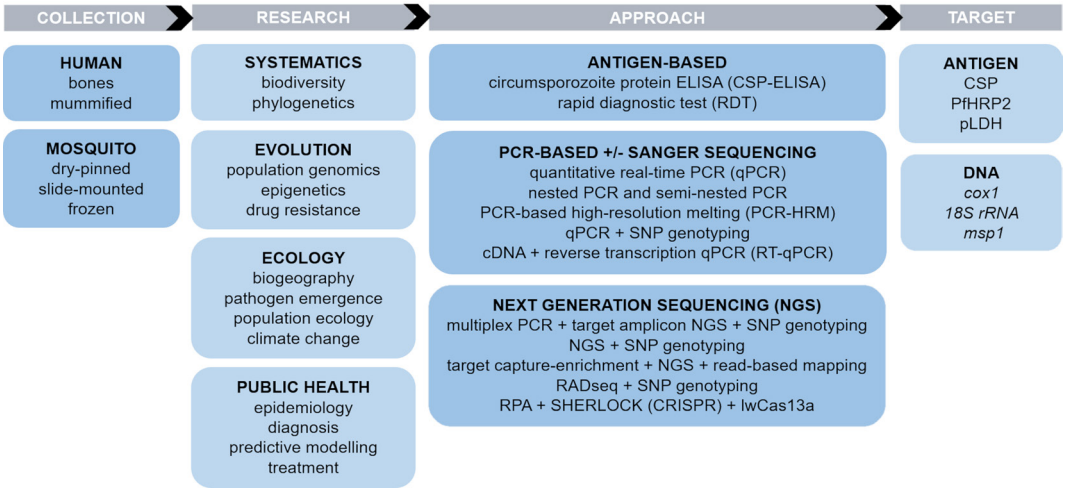

Fig. 3. Approaches to malaria research using natural history collections. *cox1*, *cytochrome c oxidase subunit 1*; CRISPR, clustered regularly interspaced short palindromic repeats; lwCas13a, *Leptotrichia wadei* CRISPR associated protein 13a; *msp1*, *merozoite surface protein 1*; PfHRP2, *Plasmodium falciparum* histidine-rich protein 2; pLDH, *Plasmodium* lactate dehydrogenase; RADseq, restriction site-associated DNA sequencing; RPA, recombinase polymerase amplification; SHERLOCK, specific high-sensitivity enzymatic reporter unlocking; SNP, single nucleotide polymorphism. Adapted from Schindel and Cook (68).

and sequencing techniques targeting *Plasmodium* mtDNA. Marciniak et al. (15) were able to detect fragments of *P. falciparum* mitogenome in two of 58 adult individuals from three different cemeteries in Italy dating to the Roman Imperial period using a *Plasmodium* species RNA-bait set. Phylogenetic analysis was unable to resolve finer evolutionary relationships within the *P. falciparum* cluster nor determine the timescale of *P. falciparum* evolution through molecular-clock dating due to only partial mitogenome reconstruction (15). Although the detection of human tissue-derived *Plasmodium* DNA can pinpoint its presence at a site at a specific time, more DNA is needed to contribute substantially to questions about the evolutionary history of the parasite, which are hampered by issues of DNA preservation and therefore consistent identification in archeological skeletons. Immunological techniques are also available for detecting *Plasmodium* DNA from ancient tissues; however, while these methods can be *Plasmodium* specific, they are not nearly as sensitive as DNA based methods (which can detect single DNA molecules) and are not able to shed light on the phylogenetic history of *Plasmodium*. Moreover, the results of these methods–when applied to archeological bones–are rather variable and complicated by related Apicomplexa species in the sediments (74). Thus, as of yet, the use of paleomicrobiology to solve questions about *Plasmodium* evolutionary history remains inconsistent. Nevertheless, paleomicrobiology tenets can be adopted to strengthen the incipient science of pathogen prospecting, including preventing contamination by modern DNA using dedicated laboratories and developing guidance on assessing the strength and authenticity of DNA evidence. Improvements in aDNA techniques offer potential for future human tissue-derived *Plasmodium* DNA and, with pathogen prospecting, reconstruct historical malaria epidemiology.

Raxworthy and Smith (82) reviewed the emerging field of museomics, the study of hDNA in archived museum collections, noting the field's importance to our understanding of evolution and pathogen emergence (83). While not anthropological, sequenced and assembled transcriptomes (RNAseq) of chipmunks collected in 1911 and 2012 provide an example of museomics in action (13). Undergoing habitat loss due to climate change, the alpine chipmunks experienced rapid evolution through shifting allele frequencies of *arachidonate 15-lipoxygenase* (*alox15*). While archived human remains (tissues, organs) in anthropological collections are sources of hDNA, most preserved tissues will likely have limited use in malaria research (84). Advances in NGS technologies will improve hDNA extraction from a variety of preserved materials, with continued development of extraction and conservation protocols (73). Studies of blood smears, as previously mentioned, are examples of how museomics aid in the reconstruction of historical disease evolution and epidemiology (14, 85). Using the same 1940s collection of slides as Gelabert et al. (14), de-Dios et al. (85) demonstrated a regionally eradicated *P. falciparum* mtDNA genome in Spain aligned more closely to Central South Asia strains than the expected African origin.

Entomological Collections. Globally, insect collections host over 0.5 billion specimens and 1 million species, offering a seemingly endless supply of material to test hypotheses on ecology, evolution, and historical epidemiology (86, 87). Selected entomological collections include Muséum d'Histoire Naturelle (40.0 million specimens; France); Smithsonian National Museum of Natural History (NMNH; 34.3 million; USA); The Natural History Museum (34.0 million; UK); Zoologische Staatssammlung München (22.8 million; Germany); National Museum of Natural History, Naturalis Biodiversity Center (17.3 million; The Netherlands); Canadian National Collection of Insects, Arachnids, and Nematodes (17.0 million; Canada); Field Museum of Natural History (16.9 million; USA); Australian National Insect Collection (13.3 million; Australia); Instituto Nacional de Pesquisas da Amazônia (4.5 million; Brazil); Lee Kong Chian Natural History Museum (1.2 million; Singapore); and KwaZulu-Natal Museum (0.5 million; South Africa) (87). The Walter Reed Biosystematics Unit at the NMNH houses the largest mosquito collection in the world at 1.7 million specimens (88). Entomological collections offer an exceptional resource to understand insect-pathogen, insect-microbiome, and insect-host relationships (Fig. 4). Recent innovations and cutting-edge techniques mean we are poised for discoveries.

Museum arthropod specimens are an underemployed, yet important, resource for understanding vector-pathogen ecology (non-malarial), disease epidemiology, and evolution, even when specimens are stored in ethanol (10, 91–94). For example, Sakamoto et al. (91) detected *Wolbachia* DNA in degraded museum bed bugs >45 y old; primers designed for short PCR amplicons overcame the issue of DNA quality. Pathogen prospecting reconstructed the historical epidemiology of Lyme disease using both vector and reservoir museum specimens. *Borrelia burgdorferi* DNA was detected in museum blacklegged ticks (*Ixodes scapularis*) collected ~30 y before the first described cases of Lyme disease in the USA (c. 1975); even earlier evidence of *B. burgdorferi* DNA comes from white-footed mice collected in MA, USA (1894) (10, 93). Phytoplasma DNA detected in museum leafhoppers (>15 y old) revealed new vector-pathogen relationships and pathogen diversity, highlighting pathogen prospecting's role as an early warning sign for disease emergence in agricultural systems (92).

Pathogen Prospecting of Museums: *Anopheles* and *Plasmodium*

At the end of the 19th century, Giovanni Grassi and colleagues, along with Ronald Ross, were the first to describe the development of *Plasmodium* in *Anopheles* and that malaria was a mosquito-borne disease (21). These discoveries led to the mainstay of detecting the infectious stage of *Plasmodium* in mosquitoes; i.e., extraction of salivary glands from live, field-caught mosquitoes and microscopic examination of Giemsa-stained sporozoites on slides (1). The sporozoite rate, or proportion of female mosquitoes with sporozoites in their salivary glands, is an entomological parameter used to assess local malaria risk. Circumsporozoite protein (CSP) ELISAs, developed in the 1980s, were used to assay freshly killed, frozen, or pinned mosquitoes for *Plasmodium* species-specific CSPs with a sensitivity of ~100 sporozoites/mosquito (95). In the early 1990s, PCR-based techniques primarily targeted the multicopy *18S rRNA* gene of *Plasmodium*, which provided a higher sensitivity of

Fig. 4. Archived *Anopheles* specimens (adult females). (*A*) Slide-mounted *Anopheles stephensi* collected in "North West Provinces India" (1910). Used with permission. Image credit: adapted from ref. 89, The Trustees of the Natural History Museum, London. CC0 1.0 (public domain). (*B*) Dry-pinned *Anopheles aquasalis* collected in "Trinidad, WI [West Indies]" (1945), with associated labels. Used with permission. Image credit: adapted from ref. 90, California Academy of Sciences. CC0 (public domain).

10 parasites/μL (96). Early parasite detection methods were designed primarily for measuring sporozoite rates in freshly collected mosquitoes, but not necessarily useful to pathogen prospecting of desiccated mosquitoes with few or degraded parasites.

Advances in the Detection of *Plasmodium* in *Anopheles* Mosquitoes. The sequencing of *P. falciparum* and *A. gambiae* genomes in 2002 ushered in the malaria genomics era, and led to sensitive molecular approaches for detecting *Plasmodium* in mosquitoes (Fig. 3) (97–102). To increase the probability of detecting *Plasmodium* DNA in pinned mosquitoes, highly sensitive techniques are needed. For instance, the fast COXI PCR assay targeting *cytochrome c oxidase 1* (*cox1*) of *Plasmodium* detects two parasites/mosquito and PCR-based high-resolution melting targeting *18S rRNA* detects one parasite/mosquito (97, 100). Few studies have addressed *Plasmodium* degradation in museum mosquitoes. Using qPCR targeting *merozoite surface protein 1* (*msp1*) DNA of *Plasmodium berghei* in desiccated *A. stephensi*, Rider et al. (99) noted that parasite DNA detection was not affected by mosquito storage for up to 6 mo at temperatures ranging from −80 °C through 28 °C. The wide temperature and storage time tolerances of *msp1*-targeting qPCR is a starting point for further baseline studies of *Plasmodium* DNA degradation in pinned mosquitoes.

Further advances in technology (e.g., CRISPR) have allowed for novel approaches of *Plasmodium* detection in *Anopheles,* including multipurpose assays (*Anopheles* and *Plasmodium* speciation, antimalarial resistance, insecticide resistance) (98, 101, 102). For example, Cunningham et al. (98) used CRISPR-based technology to identify *Plasmodium* species in field-collected anopheline mosquitoes from Thailand and detect sulfadoxine-resistant *P. falciparum*. The emergence of new approaches has potential to document *Plasmodium* genomic heterogeneity and evolution, important to understanding antimalarial resistance (similarly for *Anopheles* and insecticide resistance) (101, 102). Taken together, emerging technologies and pathogen prospecting will inform insecticide and antimalarial use and development.

While not pathogen prospecting as we have envisioned, the potential for microscopic evidence of *Plasmodium* in historical mosquito specimens (<200 y old) should not be discounted. For example, Poinar (103) detected oöcysts and sporozoites of a novel agent of avian malaria (*Plasmodium dominicana*) in a *Culex* mosquito preserved in Dominican Republic amber (c. 45 to 15 Ma.).

Taxonomic Integrity of Specimens. Pathogen prospecting should ensure preservation of a mosquito's taxonomic integrity as much as possible, as specimens are often irreplaceable vouchers and type specimens. Studies have described minimally or nondestructive mosquito DNA extraction from pinned *Anopheles* (~40 to 100 y old) using proteinase K lysis buffer, which essentially digests internal, nonchitinous tissue leaving exterior morphologies intact (104, 105). Template DNA for amplification is extracted from lysis buffer following overnight incubation of the specimen, and then the mosquito goes through several ethanol washes and is returned to the collection. Makunin et al. (106) developed a nondestructive proteinase K and lysis buffer method followed by a multilocus amplicon sequencing approach to identify freshly collected or desiccated *Anopheles* species and associated *Plasmodium* species. Another nondestructive method uses cuticular hydrocarbon profiles to detect *Plasmodium* infections and blood meals in *Anopheles albimanus*, with potential application to pinned museum specimens (107). The utility of proteinase K and cuticular hydrocarbon profiling assays to detect *Plasmodium* in museum mosquitoes remains largely unexplored.

To ensure future pathogen prospecting, we reiterate calls for archiving mosquito specimens with future minimally destructive, genomic studies in mind (87). Schindel and Cook (68) proposed Next-Generation Collections, collections that take into account an organism's parasites and symbionts, with respective preservation standards. E-vouchering has been proposed for genomic research necessitating the destruction of *Anopheles* specimens; e.g., each specimen includes a unique accession number linked to extensive diagnostic scanning electron microscopy images and mtDNA whole genome sequences (108). Thompson et al. (109) make a good argument for the early incorporation of museums into infectious disease studies and the development of best

practices for archiving the extended specimen–an organism plus its parasites and associated microbiological samples.

Limitations of Pathogen Prospecting

The ability to extract usable DNA from archived specimens is crucial to successful pathogen prospecting; however, we know little concerning the factors affecting *Plasmodium* DNA degradation and preservation in mosquitoes. *Plasmodium* DNA in dry-pinned, frozen, or slide-mounted mosquitoes will degrade over time, and depending on how they were prepared and preserved, making target DNA amplification and sequencing a challenge (Fig. 4). NGS potentially overcomes this limitation, as it captures shorter DNA templates (than amplification could) with potentially high copy numbers yields, using methods such as targeted enrichment/capture techniques and single nucleotide polymorphism genotyping (82, 86, 87). The *Plasmodium* genome is adenine-thymine rich, especially in *P. falciparum*, which complicates both amplification-based methods as well as some hybridization capture methods. Decreasing the PCR annealing temperature or designing primers for regions with higher guanine-cytosine content can help mitigate this drawback (110). Another limitation of PCR-based methods is the presence of PCR inhibitors, especially components of the mosquito cuticle, but inhibition can be overcome through repeated purifications during extraction and in some cases by use of inhibition-tolerant polymerases (97). The factors determining *Plasmodium* DNA degradation in *Anopheles* warrant baseline studies performed under a range of preservation conditions, reaction conditions, and specimen ages.

Museum collections are subject to several biases and limitations. Specimens made available to researchers will likely be nonrandom samples of convenience with selection biases, negating the possibility of statistical inferences. *Anopheles* collections are typically accrued with intent (i.e., research) and, by their nature, are accompanied by sample and collector biases, such as collecting specimens preferentially from easily accessible sites or during a specific season (111). The number of available specimens for pathogen prospecting in a mosquito biorepository typically decreases as specimen age increases (due to accumulated loss or damage, deaccessioning, disposal, damage, and DNA degradation), creating a preservation bias generally toward more contemporary specimens, but again will depend heavily on the types of preservatives used and the long-term storage conditions (i.e., dry, cool, humid, warm). Museums will not be open to wholesale destruction of specimens; however, may be amenable to disposing of mosquitoes using a priori specimen selection criteria. This final limitation emphasizes the importance of early interdisciplinary collaboration with museum curators, researchers, and collections staff to ensure a proposed project meets a museum's mission.

Conclusions

The integration of pathogen prospecting into existing disease surveillance programs is an opportunity to develop public health interventions to lessen the global burden of malaria. The recent COVID-19 pandemic highlighted the need for proactive pathogen discovery and surveillance programs, integrated into public health infrastructure (112–114). Pathogen prospecting for additional vector-pathogen dyads promises a broader understanding of infectious disease epidemiology and pathogen evolution. For malaria, pathogen prospecting will:

- provide a snapshot of historical malaria epidemiology;
- generate spatiotemporal data that will improve predictive modeling of malaria risk;
- elucidate evolutionary change in *Plasmodium* and *Anopheles*;
- inform bioinformatics and development of insecticides, diagnostics, therapeutics, and vaccines; and
- initiate the development of standardized protocols for mosquito preservation and archiving, thereby, minimizing DNA degradation and ensuring sustainable pathogen prospecting.

Undoubtedly, we have not exhausted all possibilities and limitations of pathogen prospecting. Our Perspective should encourage further discussions on the future of pathogen prospecting, prompting collaborative, transdisciplinary studies.

Data, Materials, and Software Availability. There are no data underlying this work.

ACKNOWLEDGMENTS. We thank Wade Hemsworth (McMaster University) for helping prepare our submission and two anonymous reviewers for their insightful comments to improve the manuscript. This research was undertaken, in part, thanks to funding from the Canada Research Chairs program (Grant/Award Number: 231563), the Dutch Research Council (NWO) (grant reference: 016.Veni.195.195), National Sciences and Engineering Research Council of Canada (Grant number: 10539150), and Elise Mathilde Fund/Leiden University Fund (grant reference: W18357-6-EM).

1. World Health Organization, *World Malaria Report 2023* (World Health Organization, 2023).
2. G. L. Miller, Historical natural history: Insects and the civil war. *Am. Entomol.* **43**, 227–245 (1997).
3. E. G. Tse, M. Korsik, M. H. Todd, The past, present and future of anti-malarial medicines. *Malar. J.* **18**, 93 (2019).
4. E. T. Piperaki, G. L. Daikos, Malaria in Europe: Emerging threat or minor nuisance? *Clin. Microbiol. Infect.* **22**, 487–493 (2016).
5. Centers for Disease Control and Prevention, Locally acquired cases of malaria in Florida, Texas, Maryland, and Arkansas (2023). https://www.cdc.gov/malaria/new_info/2023/malaria_US.html (Accessed 8 November 2023).
6. D. Blackburn *et al.*, Outbreak of locally acquired mosquito-transmitted (autochthonous) malaria - Florida and Texas, May–July 2023. *MMWR Morb. Mortal. Wkly. Rep.* **72**, 973–978 (2023).
7. S. I. Hay, C. A. Guerra, A. J. Tatem, A. M. Noor, R. W. Snow, The global distribution and population at risk of malaria: Past, present, and future. *Lancet Infect. Dis.* **4**, 327–336 (2004).
8. D. Chandramohan *et al.*, Seasonal malaria vaccination with or without seasonal malaria chemoprevention. *N. Engl. J. Med.* **385**, e035433 (2021).
9. V. S. Balakrishnan, WHO recommends malaria vaccine for children. *Lancet Infect. Dis.* **21**, 1634 (2021).
10. D. H. Persing *et al.*, Detection of *Borrelia burgdorferi* DNA in museum specimens of *Ixodes dammini* ticks. *Science* **249**, 1420–1423 (1990).
11. S. Haensch *et al.*, Distinct clones of *Yersinia pestis* caused the black death. *PLoS Pathog.* **6**, e1001134 (2010).
12. A. Loufouma Mbouaka *et al.*, The elusive parasite: Comparing macroscopic, immunological, and genomic approaches to identifying malaria in human skeletal remains from Sayala, Egypt (third to sixth centuries AD). *Archaeol. Anthropol. Sci.* **13**, 115 (2021).
13. K. Bi *et al.*, Temporal genomic contrasts reveal rapid evolutionary responses in an alpine mammal during recent climate change. *PLoS Genet.* **15**, e1008119 (2019).
14. P. Gelabert *et al.*, Mitochondrial DNA from the eradicated European *Plasmodium vivax* and *P. falciparum* from 70-year-old slides from the Ebro Delta in Spain. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 11495–11500 (2016).

15. S. Marciniak *et al.*, *Plasmodium falciparum* malaria in 1st–2nd century CE southern Italy. *Curr. Biol.* **26**, R1220–R1222 (2016).
16. E. E. Enabulele *et al.*, Prospecting for zoonotic pathogens by using targeted DNA enrichment. *Emerg. Infect. Dis.* **29**, 1566–1579 (2023).
17. M. Shpak, H. R. Ghanavi, J. D. Lange, J. E. Pool, M. C. Stensmyr, Genomes from historical *Drosophila melanogaster* specimens illuminate adaptive and demographic changes across more than 200 years of evolution. *PLoS Biol.* **21**, e3002333 (2023).
18. S. Ringer, On the connexion between the heat of the body and the excreted amounts of urea, chloride of sodium, and urinary water, during a fit of ague. *Med. Chir. Trans.* **42**, 361–401 (1859).
19. J. Bartlett, S. Ringer, Two cases of ague, with a few remarks on the same. *Edinb. Med. J.* **10**, 303–306 (1864).
20. C. W. Duggan, The parasite of malaria in the fevers of West Africa. *Br. Med. J.* **1**, 139–140 (1898).
21. F. E. G. Cox, History of the discovery of the malaria parasites and their vectors. *Parasit. Vectors* **3**, 5 (2010).
22. J. MacCulloch, *Malaria: An Essay on the Production and Propagation of this Poison, and on the Nature and Localities of the Places by which it is Produced; with an Enumeration of the Diseases Caused by it, and of the Means of Preventing or Diminishing them, both at Home and in the Naval and Military Service* (Longman, Rees, Orme, Brown, and Green, 1827).
23. C. A. Gordon, The hygiene of malaria. *Ind. Med. Gaz.* **9**, 191–194 (1874).
24. W. J. Moore, Malaria vs. recognisable climatic influences. *Ind. Med. Gaz.* **16**, 293–306 (1881).
25. L. J. Bruce-Chwatt, J. M. Bruce-Chwatt, Malaria in Mauritius–as dead as the dodo. *Bull. N. Y. Acad. Med.* **50**, 1069–1080 (1974).
26. G. Bennett, Account of the intermittent fever, as it prevailed among a native gang at the Island of Erromanga, one of the New Hebrides Group. *Lond. Med. Phys. J.* **12**, 175–182 (1832).
27. M. Oxenham, H. Buckley, *The Routledge Handbook of Bioarchaeology in Southeast Asia and the Pacific Islands* (Routledge, 2015).
28. H. R. Buckley, "'The predators within': Investigating the relationship between malaria and health in the prehistoric Pacific Islands" in *Bioarchaeology of Southeast Asia*, M. Oxenham, N. Tayles, Eds. (Cambridge University Press, 2006), pp. 309–332.
29. P. H. Adler, W. Wills, Legacy of death: The history of arthropod-borne human diseases in South Carolina. *Am. Entomol.* **49**, 216–228 (2003).
30. G. Dods, Tropical malaria and its sequelæ. *Trans. Medi. Chir Soc. Edinb.* **33**, 1090–1099 (1888).
31. S. C. Hong, The burden of early exposure to malaria in the United States, 1850–1860: Malnutrition and immune disorders. *J. Econ. Hist.* **67**, 1001 (2007).
32. J. Sachs, P. Malaney, The economic and social burden of malaria. *Nature* **415**, 680–685 (2002).
33. M. V. Andrade *et al.*, The economic burden of malaria: A systematic review. *Malar. J.* **21**, 283 (2022).
34. C. A. Bentley, Some economic aspects of Bengal malaria. *Ind. Med. Gaz.* **57**, 321–326 (1922).
35. P. W. Hedrick, Population genetics of malaria resistance in humans. *Heredity* **107**, 283–304 (2011).
36. S. N. Kariuki, T. N. Williams, Human genetics and malaria resistance. *Hum. Genet.* **139**, 801–811 (2020).
37. E. M. Leffler *et al.*, Resistance to malaria through structural variation of red blood cell invasion receptors. *Science.* **356**, eaam6393 (2017).
38. T. N. Williams, Human red blood cell polymorphisms and malaria. *Curr. Opin. Microbiol.* **9**, 388–394 (2006).
39. T. S. Athni *et al.*, The influence of vector-borne disease on human history: Socio-ecological mechanisms. *Ecol. Lett.* **24**, 829–846 (2021).
40. L. M. R. Nasim, "Shaping colonial science: Missionary challenges, racial segregation and the locality of science" in *Medical Missionaries and Colonial Knowledge in West Africa and Europe, 1885–1914*, L. M. R. Nasim, Ed. (Palgrave Macmillan, Cham, 2023), pp. 313–346.
41. F. J. Colón-González *et al.*, Projecting the risk of mosquito-borne diseases in a warmer and more populated world: A multi-model, multi-scenario intercomparison modelling study. *Lancet Planet Health* **5**, E404–E414 (2021).
42. R. E. Fontaine, A. E. Najjar, J. S. Prince, The 1958 malaria epidemic in Ethiopia. *Am. J. Trop. Med. Hyg.* **10**, 795–803 (1961).
43. P. V. V. Le, P. Kumar, M. O. Ruiz, C. Mbogo, E. J. Muturi, Predicting the direct and indirect impacts of climate change on malaria in coastal Kenya. *PLoS One* **14**, e0211258 (2019).
44. C. J. Carlson, E. Bannon, E. Mendenhall, T. Newfield, S. Bansal, Rapid range shifts in African mosquitoes over the last century. *Biol. Lett.* **19**, 20220365 (2023).
45. E. Hertig, Distribution of *Anopheles* vectors and potential malaria transmission stability in Europe and the Mediterranean area under future climate change. *Parasit. Vectors* **12**, 18 (2019).
46. L. McIver *et al.*, Health impacts of climate change in Pacific island countries: A regional assessment of vulnerabilities and adaptation priorities. *Environ. Health Perspect.* **124**, 1707–1714 (2016).
47. G. Robbins Schug *et al.*, Climate change, human health, and resilience in the Holocene. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2209472120 (2023).
48. C. Caminade *et al.*, Impact of climate change on global malaria distribution. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 3286–3291 (2014).
49. J. L. Espinoza, Malaria resurgence in the Americas: An underestimated threat. *Pathogens* **8**, 11 (2019).
50. I. K. Fletcher *et al.*, Synergies between environmental degradation and climate variation on malaria re-emergence in southern Venezuela: A spatiotemporal modelling study. *Lancet Planet Health* **6**, e739–e748 (2022).
51. A. J. MacDonald, E. A. Mordecai, Amazon deforestation drives malaria transmission, and malaria burden reduces forest clearing. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 22212–22218 (2019).
52. J. Rocklöv, R. Dubrow, Climate change: An enduring challenge for vector-borne disease prevention and control. *Nat. Immunol.* **21**, 479–483 (2020).
53. R. E. Baker *et al.*, Infectious disease in an era of global change. *Nat. Rev. Microbiol.* **20**, 193–205 (2021).
54. S. H. Olson, R. Gangnon, G. A. Silveira, J. A. Patz, Deforestation and malaria in Mâncio Lima County, Brazil. *Emerg. Infect. Dis.* **16**, 1108–1115 (2010).
55. M. L. Wilson *et al.*, Urban malaria: Understanding its epidemiology, ecology, and transmission across seven diverse ICEMR network sites. *Am. J. Trop. Med. Hyg.* **93**, 110–123 (2015).
56. M. E. Sinka *et al.*, A new malaria vector in Africa: Predicting the expansion range of *Anopheles stephensi* and identifying the urban populations at risk. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 24900–24908 (2020).
57. P. Doumbe-Belisse *et al.*, Urban malaria in sub-Saharan Africa: Dynamic of the vectorial system and the entomological inoculation rate. *Malar. J.* **20**, 364 (2021).
58. G. Z. Laporta *et al.*, Malaria transmission in landscapes with varying deforestation levels and timelines in the Amazon: A longitudinal spatiotemporal study. *Sci. Rep.* **11**, 6477 (2021).
59. S. M. Feleke *et al.*, *Plasmodium falciparum* is evolving to escape malaria rapid diagnostic tests in Ethiopia. *Nat. Microbiol.* **6**, 1289–1299 (2021).
60. M. D. Conrad, P. J. Rosenthal, Antimalarial drug resistance in Africa: The calm before the storm? *Lancet Infect. Dis.* **19**, e338–e351 (2019).
61. M. H. Nyunt *et al.*, Molecular assessment of artemisinin resistance markers, polymorphisms in the K13 propeller, and a multidrug-resistance gene in the eastern and western border areas of Myanmar. *Clin. Infect. Dis.* **60**, 1208–1215 (2014).
62. S. Newey, War-torn Myanmar hit by 1,000pc leap in malaria cases (The Sunday Telegraph, 2023). https://www.telegraph.co.uk/global-health/science-and-disease/myanmar-malaria-surge-upturn-eradication-mekong-delta/. Accessed 8 March 2024.
63. D. E. Loy *et al.*, Evolutionary history of human *Plasmodium vivax* revealed by genome-wide analyses of related ape parasites. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E8450–E8459 (2018).
64. R. M. Packard, The origins of antimalarial-drug resistance. *N. Engl. J. Med.* **371**, 397–399 (2014).
65. M. F. Suarez, M. L. Quiñones, J. D. Palacios, A. Carrillo, First record of DDT resistance in *Anopheles darlingi*. *J. Am. Mosq. Control Assoc.* **6**, 72–74 (1990).
66. I. Hamid, K. L. Korunes, S. Beleza, A. Goldberg, Rapid adaptation to malaria facilitated by admixture in the human population of Cabo Verde. *Elife* **10**, e63177 (2021).
67. A. V. Suarez, N. D. Tsutsui, The value of museum collections for research and society. *Bioscience* **54**, 66–74 (2004).
68. D. E. Schindel, J. A. Cook, The next generation of natural history collections. *PLoS Biol.* **16**, e2006125 (2018).
69. J. Giblin, I. Ramos, N. Grout, Dismantling the master's house. *Third Text* **33**, 471–486 (2019).
70. M. Prösler, Museums and globalization. *Sociol. Rev.* **43**, 21–44 (1995).
71. C. Warinner *et al.*, A robust framework for microbial archaeology. *Annu. Rev. Genomics Hum. Genet.* **18**, 321–356 (2017).
72. S. Duchêne, S. Y. W. Ho, A. G. Carmichael, E. C. Holmes, H. Poinar, The recovery, interpretation and use of ancient pathogen genomes. *Curr. Biol.* **30**, R1215–R1231 (2020).
73. C. Lalueza-Fox, Museomics. *Curr. Biol.* **32**, R1214–R1215 (2022).
74. R. Schats, Developing an archaeology of malaria. A critical review of current approaches and a discussion on ways forward. *Int. J. Paleopath.* **41**, 32–42 (2023).
75. M. B. Brickley, Perspectives on anemia: Factors confounding understanding of past occurrence. *Int. J. Paleopath.* **44**, 90–104 (2024).
76. R. L. Gowland, A. G. Western, Morbidity in the marshes: Using spatial epidemiology to investigate skeletal evidence for Malaria in Anglo-Saxon England (AD 410–1050). *Am. J. Phys. Anthropol.* **147**, 301–311 (2012).
77. R. Schats, Malaise and mosquitos: Osteoarchaeological evidence for malaria in the medieval Netherlands. *Analecta Praehist. Leiden.* **45**, 133–140 (2015).
78. M. E. Lewis, Thalassaemia: Its diagnosis and interpretation in past skeletal populations. *Int. J. Osteoarchaeol.* **22**, 685–693 (2012).
79. M. Vlok *et al.*, Forager and farmer evolutionary adaptations to malaria evidenced by 7000 years of thalassemia in Southeast Asia. *Sci. Rep.* **11**, 1–15 (2021).
80. I. Hershkovitz *et al.*, Recognition of sickle cell anemia in skeletal remains of children. *Am. J. Phys. Anthropol.* **104**, 213–226 (1997).
81. C. D. Matheson *et al.*, Molecular exploration of the first-century Tomb of the Shroud in Akeldama, Jerusalem. *PLoS One* **4**, e8319 (2009).
82. C. J. Raxworthy, B. T. Smith, Mining museums for historical DNA: Advances and challenges in museomics. *Trends Ecol. Evol.* **36**, 1049–1060 (2021).
83. M. S. Tiee, R. J. Harrigan, H. A. Thomassen, T. B. Smith, Ghosts of infections past: Using archival samples to understand a century of monkeypox virus prevalence among host communities across space and time. *R. Soc. Open Sci.* **5**, 171089 (2018).
84. A. M. Devault *et al.*, Second-pandemic strain of *Vibrio cholerae* from the Philadelphia cholera outbreak of 1849. *N. Engl. J. Med.* **370**, 334–340 (2014).
85. T. de-Dios *et al.*, Genetic affinities of an eradicated European *Plasmodium falciparum* strain. *Microb. Genom.* **5**, e000289 (2019).
86. M. Staats *et al.*, Genomic treasure troves: Complete genome sequencing of herbarium and insect museum specimens. *PLoS One* **8**, e69189 (2013).
87. A. E. Z. Short, T. Dikow, C. S. Moreau, Entomological collections in the age of big data. *Annu. Rev. Entomol.* **63**, 513–530 (2018).
88. Smithsonian National Museum of Natural History, Entomology (2023), https://naturalhistory.si.edu/research/entomology (Accessed 26 December 2023).
89. The Trustees of the Natural History Museum, London, Data portal (2023), https://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb/record/9791828 (Accessed 20 December 2023).
90. California Academy, of Sciences, Monarch (2023), https://monarch.calacademy.org/taxa/index.php?taxon=697272 (Accessed 20 December 2023).

91. J. M. Sakamoto, J. Feinstein, J. L. Rasgon, *Wolbachia* infections in the Cimicidae: Museum specimens as an untapped resource for endosymbiont surveys. *Appl. Environ. Microbiol.* **72**, 3161–3167 (2006).
92. V. Trivellone, W. Wei, L. Filippin, C. H. Dietrich, Screening potential insect vectors in a museum biorepository reveals undiscovered diversity of plant pathogens in natural areas. *Ecol. Evol.* **11**, 6493–6503 (2021).
93. W. F. Marshall *et al.*, Detection of *Borrelia burgdorferi* DNA in museum specimens of *Peromyscus leucopus*. *J. Infect. Dis.* **170**, 1027–1032 (1994).
94. S. N. R. Brasil, E. P. Kelemen, S. M. Rehan, Historic DNA uncovers genetic effects of climate change and landscape alteration in two wild bee species. *Conserv. Genet.* **24**, 85–98 (2022).
95. R. A. Wirtz, T. R. Burkot, P. M. Graves, R. G. Andre, Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *J. Med. Entomol.* **24**, 433–437 (1987).
96. G. Snounou *et al.*, High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Mol. Biochem. Parasitol.* **61**, 315–320 (1993).
97. D. F. Echeverry *et al.*, Fast and robust single PCR for *Plasmodium* sporozoite detection in mosquitoes using the cytochrome oxidase I gene. *Malar. J.* **16**, 230 (2017).
98. C. H. Cunningham *et al.*, A novel CRISPR-based malaria diagnostic capable of *Plasmodium* detection, species differentiation, and drug-resistance genotyping. *EBioMedicine* **68**, 103415 (2021).
99. M. A. Rider, B. D. Byrd, J. Keating, D. M. Wesson, K. A. Caillouet, PCR detection of malaria parasites in desiccated *Anopheles* mosquitoes is uninhibited by storage time and temperature. *Malar. J.* **11**, 193 (2012).
100. E. Murillo, C. Muskus, L. A. Agudelo, I. D. Vélez, F. Ruiz-Lopez, A new high-resolution melting analysis for the detection and identification of *Plasmodium* in human and *Anopheles* vectors of malaria. *Sci. Rep.* **9**, 1674 (2019).
101. D. E. Neafsey, A. R. Taylor, B. L. MacInnis, Advances and opportunities in malaria population genomics. *Nat. Rev. Genet.* **22**, 502–517 (2021).
102. M. Campos *et al.*, High-throughput barcoding method for the genetic surveillance of insecticide resistance and species identification in *Anopheles gambiae* complex malaria vectors. *Sci. Rep.* **12**, 13893 (2022).
103. G. Poinar, *Plasmodium dominicana* n. sp. (Plasmodiidae: Haemosporida) from Tertiary Dominican amber. *Syst. Parasitol.* **61**, 47–52 (2005).
104. P. Korlević *et al.*, A minimally morphologically destructive approach for DNA retrieval and whole-genome shotgun sequencing of pinned historic dipteran vector species. *Genome Biol. Evol.* **13**, evab226 (2021).
105. I. A. Giantsis, A. Chaskopoulou, M. C. Bon, Mild-vectolysis: A nondestructive DNA extraction method for vouchering sand flies and mosquitoes. *J. Med. Entomol.* **53**, 692–695 (2016).
106. A. Makunin *et al.*, A targeted amplicon sequencing panel to simultaneously identify mosquito species and *Plasmodium* presence across the entire *Anopheles* genus. *Mol. Ecol. Resour.* **22**, 28–44 (2021).
107. F. Claudio-Piedras *et al.*, Dietary and *Plasmodium* challenge effects on the cuticular hydrocarbon profile of *Anopheles albimanus*. *Sci. Rep.* **11**, 11258 (2021).
108. S. A. Justi *et al.*, From e-voucher to genomic data: Preserving archive specimens as demonstrated with medically important mosquitoes (Diptera: Culicidae) and kissing bugs (Hemiptera: Reduviidae). *PLoS One* **16**, e0247068 (2021).
109. C. W. Thompson *et al.*, Preserve a voucher specimen! The critical need for integrating natural history collections in infectious disease studies. *MBio* **12**, e02698–e02720 (2021).
110. M. J. López-Barragán *et al.*, Effect of PCR extension temperature on high-throughput sequencing. *Mol. Biochem. Parasitol.* **176**, 64–67 (2011).
111. E. K. Meineke, B. H. Daru, Bias assessments to expand research harnessing biological collections. *Trends Ecol. Evol.* **36**, 1071–1082 (2021).
112. J. B. Nachega *et al.*, Advancing detection and response capacities for emerging and re-emerging pathogens in Africa. *Lancet Infect. Dis.* **23**, e185–e189 (2023).
113. J. A. Cook *et al.*, Integrating biodiversity infrastructure into pathogen discovery and mitigation of emerging infectious diseases. *BioScience* **70**, 531–534 (2020).
114. S. C. Inzaule, S. K. Tessema, Y. Kebede, A. E. O. Ouma, J. N. Nkengasong, Genomic-informed pathogen surveillance in Africa: Opportunities and challenges. *Lancet Infect. Dis.* **21**, e281–e289 (2021).