Tiny travel companions

As microorganisms have accompanied mankind's journeys around the globe, they could help scientists to unravel our past

Fig 1 | Map of early human migration patterns. Credit: National Geographic Maps, Washington, DC, USA. mtDNA, mitochondrial DNA.

Since the first modern humans left their birthplace in Eastern Africa and slowly spread to populate every inhabitable place on the planet, *Homo sapiens* has been a migratory animal. Over thousands of years, these travellers unknowingly carried excess baggage on their journeys: countless microbes in and on their bodies, which evolved along with their host. By applying the tools of modern genomics to reconstruct the phylogenetic trees of these viruses, bacteria, fungi and protozoa, scientists hope to provide

new insights into how our ancestors ventured throughout the world. Answering the questions of when and how humans started moving out of Africa or when the first settlers populated the Americas has so far relied on genetic markers from mitochondrial DNA (mtDNA) or the Y chromosome. As mitochondria are inherited from the mother, analysis of mtDNA identifies ancestors through the maternal line, whereas Y-DNA can trace origins using the paternal line. These studies, together with

archaeological, anthropological and linguistic evidence, have generated a now widely accepted “standard model for evolution of modern humans” (Cavalli-Sforza & Feldman, 2003). According to this model, our species moved from its original homeland in Africa to the Near East approximately 60,000–70,000 years ago, later spreading to southern Asia and Australasia, then to Europe and Central Asia, and finally entered the Americas through the Bering Strait about 14,000–20,000 years ago (Fig 1).

Fig 2 | Electron micrograph of *Helicobacter pylori*, with several flagella (negative staining). This species is one of the best candidates for tracing human migrations. Credit: Wikipedia Commons.

In addition to these human markers, scientists have begun to study the evolution of viruses along with their human hosts to refine the migration patterns of *H. sapiens*. “The geographical distribution of viruses can reveal patterns of ancient human migrations, especially in the cases in which they are transmitted vertically and move in narrow, ethnically restricted streams of infection,” said Angelo Pavesi, a population geneticist at the University of Parma, Italy. “The main benefit is that viruses show a more striking genetic variation with respect to human genes. Therefore, they can provide novel clues on the history of our ancestors, rather than a pure replication of the pattern yielded by human genes.”

In particular, Pavesi pointed out the single-stranded RNA hepatitis G virus and the double-stranded DNA JC polyomavirus (JCV). Phylogenetic studies based on genomic data indicated an African origin for the hepatitis G virus, and showed that isolates from Southeast Asia are most closely related to the African strains. This finding would support the model of one major human migration from Africa to Southeast Asia, indicated by studies of human mitochondrial haplotypes.

However, another scenario based on studies of JCV suggests that an ancestral strain gave rise to two distinct evolutionary lineages, which left Africa along the Levantine route and then diffused into the rest of the world (Pavesi, 2005a). “This latter finding suggests that the expansion of our

ancestors from Africa was mediated by two distinct migration waves, each carrying a different JCV lineage,” said Pavesi. “If correct, this hypothesis sheds new light on the pattern of human migrations yielded so far by human genes, which supports the view of one single expansion from Africa into Asia and from there to the other continents.”

Both hepatitis G and JCV have some features that make them potentially good markers of human history. The hepatitis virus virtually lacks pathogenicity and is transferred vertically from mother to infant through breastfeeding. JCV, which seems to be an even better tracer for human history, is ubiquitous in human populations throughout the world with a seroprevalence of 70–90%, and is transmitted in a quasi-vertical manner. Infections are usually benign—the virus causes progressive multifocal leukoencephalopathy in a small percentage of severely immunocompromised patients—and it rarely undergoes genetic recombination. Other potential viral markers include human T-cell lymphotropic virus, human papillomavirus and even HIV (Wirth *et al*, 2005; Pavesi, 2005b).

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But these viral hitchhikers also have some drawbacks with regard to tracing their travels with their human hosts. Hepatitis G has a low infection rate—from 1–4% in industrialized countries to 10–13% in developing countries—which means that it is not widespread in populations and is thus difficult to isolate. Concurrent horizontal transmission by sexual contact or blood transfusion, and the possibility of recombination between genetically different strains,

can further obscure phylogenetic relationships between geographically distant isolates (Wirth *et al*, 2005; Pavesi, 2005b).

JCV has also come under scrutiny recently, with research casting some doubts on its utility as a marker of human migrations. A group led by Edward Holmes at Pennsylvania State University (University Park, PA, USA) used a large viral genome data set to re-analyse the distribution of JCV subtypes in human populations, and to estimate the evolutionary rate of the virus (Shackelton *et al*, 2006). They found a number of significant discrepancies between viral subtypes and specific human populations, which challenge the view that JCV has co-evolved with humans since their emergence from Africa. For example, some isolates from Japanese and Korean people are similar to strains found among Europeans. Even more remarkable is that although the most genetically divergent and therefore ancient human populations are from Africa, the greatest diversity in JCV seems to be in Europe, which suggests that the virus originally evolved from early Europeans.

“Strikingly, our comparisons of JCV and human phylogenies provided no evidence for codivergence,” wrote Holmes and colleagues (Shackelton *et al*, 2006). “As such, we caution against using this virus to make extensive inferences about the evolution of human populations.” Providing further support for this view, Holmes and his team estimated a rapid evolutionary rate for JCV, significantly higher than that underlying the model of co-evolution in which the host and microbe evolve at comparable speeds (Shackelton *et al*, 2006).

“In light of this study, which proposes an origin of JCV dating back 310–3100 years, further investigations on the evolution of this viral marker are an obligate step,” conceded Pavesi. “If correct, this estimate does prevent any inference about our past history.” However, he maintains that the worldwide diffusion of JCV, especially if mediated by a slow mechanism of vertical transmission from generation to generation, cannot have occurred in such a small period of time. “An adequate response could be an accurate examination of the available sequence data, with the aim to assess the validity of the generally accepted evolutionary pattern [the African origin of JCV and differentiation of two distinct lineages from the ancestral strain] and to provide additional estimates of the time of emergence/divergence of the virus,” Pavesi said.

In the meantime, another promising candidate with which to study human migrations has emerged: *Helicobacter pylori*. This Gram-negative bacterium colonizes the stomach of approximately half the human population, and is associated with gastric and duodenal ulcers, although in most cases it lives commensally without causing any clinical symptoms (Fig 2). It recombines frequently, but as transmission occurs predominantly within families, mutations tend to accumulate in a local bacterial population, thus forming a pool of relatively homogeneous strains. As a result, isolates of *H. pylori* can be divided into different populations and subpopulations with distinct geographical distributions.

Reconstructing the phylogenetic relationships of these clusters on a global scale, starting from five ancestral populations identified by genetic linkage analysis, resulted in a pattern of human migration and expansion similar to that obtained by other genetic tools or by archaeological and historical evidence (Falush *et al*, 2003). Recent studies also confirmed that, when examined in greater detail, *H. pylori* strains can be used to distinguish between closely related human populations more accurately than human genetic markers. To test this hypothesis, biologists used the distribution of *H. pylori* strains to trace the recent history of people in Ladakh—an isolated Indian province in the trans-Himalayan region where Buddhists and Muslims have lived together for centuries, but rarely mixed (Wirth *et al*, 2004). The population genetic structure of *H. pylori* from the two communities differs in a way that fits the known human history of the region, revealing the contribution of migratory episodes, genetic fluxes from adjacent areas and even missionaries. “*H. pylori* sequence analysis has the potential to become an important tool for unraveling short-term genetic changes in human populations,” the authors concluded, remarking that microsatellites or mtDNA are not suitable for detecting very recent admixtures. “*H. pylori* is more than a proxy measure of human ancestry and might even be considered to represent one of the most

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Fig 3 | Dissemination of leprosy in the world, based on the analysis of single-nucleotide polymorphisms. The ovals indicate the country of origin of the samples examined and their distribution into four SNP types: yellow, type 1; orange, type 2; pink, type 3; green, type 4. The coloured arrows indicate the direction of human migrations predicted by, or inferred from SNP analysis; white dotted arrows correspond to the migration routes of humans derived from genetic, archaeological and anthropological studies, with the estimated time of migration in years. From Monot *et al* (2005). Reproduced with permission from AAAS, Washington, DC, USA. SNP, single-nucleotide polymorphisms.

promising alternatives to classical human genetic markers for analyzing introgression between human populations” (Wirth *et al*, 2004).

As human history is so intimately intertwined with the evolution of our pathogens, the study of microbial genetic markers can also be used to understand how human migration fostered disease dissemination. A research group led by molecular microbiologist Stewart Cole at the Pasteur Institute in Paris, France, has recently shed light on the origin and spread of leprosy (Monot *et al*, 2005). This chronic infection of the skin and peripheral nerves has plagued every continent for millennia, and left its indelible mark in each culture it visited. Even in areas where it has long disappeared, a mix of terrifying images of disfiguration, mutilation and torment subconsciously persists. Traditionally, the scourge of leprosy was thought to have originated in India and later introduced to Europe by Alexander the Great’s soldiers returning from their campaign a few years before the death of their commander in 323 BC.

The causative agent of leprosy, *Mycobacterium leprae*, is an obligate intracellular bacterium that has undergone

massive genome reduction while adapting to its host. By using a comparative genomics approach, Cole and his colleagues first showed that *M. leprae* has very low genetic diversity, to the degree that all extant cases of leprosy seem attributable to a single clone, which apparently infected the entire world. The researchers then looked at single-nucleotide polymorphisms in 175 strains from all over the world and identified four types whose current distribution offered the basis for tracing the evolutionary path of the disease. On these grounds, leprosy should have arisen in East Africa or the Near East, and then spread to Europe and Asia. Colonialism, emigration and even the slave trade successively contributed to the introduction of leprosy into West Africa and the Americas over the past 500 years (Fig 3). “Owing to its remarkably stable genome, where polymorphisms are exceptionally rare compared to other bacterial pathogens, the leprosy bacillus is a very useful marker for following human migrations,” Cole commented.

Another interesting example is *Coccidioides immitis*, a highly virulent soil fungus endemic in the Americas, where it causes the potentially fatal disease coccidioidomycosis (Hector & Laniado-Laborin,

OF LICE AND MEN

Although humans are a recently evolved species, to which relatively few parasites have adapted, several species can be used for reconstructing human evolutionary history because they can provide information independent of their host (Ashford, 2000). Head lice, *Pediculus humanus* (shown here), for example, have a lot to say. A research team at the University of Florida (Gainesville, USA) used morphological and molecular data to investigate the evolutionary history of *P. humanus*, dating the origin of the human louse and calculating divergence dates for nodes in the louse phylogeny (Reed *et al*, 2004). This approach showed that head lice contain two ancient lineages, one of which has a worldwide distribution, whereas the other is restricted to the New World and diverged from the former at least 1.18 million years ago. The parasite thus originated long before its present *Homo sapiens* host, who probably arose in Africa from more ancient forms only about 130,000 years ago. Results suggest that the two identified louse lineages originally co-evolved and co-diverged with early species of *Homo* that were already present on several continents. However, although the worldwide clade of lice co-evolved with predecessors of modern *H. sapiens*, the New World clade probably parasitized *H. erectus*, and switched to *H. sapiens* only recently (as shown below). “[T]his implies that *H. erectus* was contemporaneous with modern *H. sapiens* in eastern Asia,” the authors concluded, from where our ancestors colonized the Americas, and that the two species were physically close enough—although not necessarily through sexual contact—for archaic lice to jump on modern human hosts (Reed *et al*, 2004).

Dorsal view of a female head louse, *Pediculus humanus capitis*. Credit: Centers for Disease Control and Prevention, Atlanta, GA, USA; Dennis D. Juraneck.

Inspecting human evolution by using lice can also reveal unexpected details of how specific elements of our culture were acquired. Ralf Kittler and colleagues at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, looked at the origin of the body louse (Kittler *et al*, 2003). This subspecies, *P. humanus corporis*—the vector of the epidemic typhus, relapsing fever, trench fever and other diseases—feeds on the body but lives in clothing, thus differing from the head louse. By performing a molecular clock analysis of mtDNA from both head and body lice, the researchers showed that the body louse evolved from the head louse not more than about $72,000 \pm 42,000$ years ago. “These results suggest that clothing was a surprisingly recent innovation in human evolution,” wrote the authors, assuming that the emergence of a separate branch in human louse genealogy reflects the appearance of a new ecological niche when humans first made frequent use of clothing, an event for which there is no direct archaeological evidence (Kittler *et al*, 2003). “Indeed, clothing may have allowed early modern humans to colonize more extreme latitudes than their archaic predecessors, and hence might have been a factor in the successful spread of modern humans out of Africa.”

(left) Temporal and geographical distribution of hominid populations. The figure depicts one view of human evolutionary history based on fossil data. The temporal distribution of the two divergent lineages of *Pediculus humanus* is superimposed on the hominid tree to show host evolutionary events that were contemporaneous with the origin of *P. humanus*. The New World lineage (black) is depicted on *Homo erectus*, whereas the worldwide clade (red) leads directly to *H. sapiens*. Fossil evidence indicate that *H. erectus* disappeared as late as 50,000 years ago. Reproduced from Reed *et al* (2004).

2005). By using microsatellite analysis of isolates from North, Central and South America, a study of the pathogen’s population genetic structure suggested that *C. immitis* entered South America—where it is now prevalent—from the north during the late Pleistocene era, probably carried by a mammalian host (Fisher *et al*, 2001).

Among the various possibilities, the timing and pattern of its dispersal coincides with the postulated spread of humans on the American continent. In particular, archaeological evidence specifically links this fungus to Amerindian people, who are known to have migrated to South America at least 12,500 years ago.

Although the evidence from co-evolving microbes seems to support the general theory of how humans moved from Africa to the rest of the world, many details of this saga are not yet clear. For example, the discussion of how many waves of humans crossed into the Americas, and where they migrated once there, continues. To help

answer questions such as this, the National Geographic Society (Washington, DC, USA) and IBM (Armonk, NY, USA) created the Genographic Project, a five-year research initiative that will trace the migratory history of humans (www.nationalgeographic.com/genographic/). Under the scientific supervision of population geneticist Spencer Wells, the project will collect mtDNA and Y-DNA data from more than 100,000 indigenous people around the world and an equal number from the general population.

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Other attempts to reconstruct human migrations try to piece together information from additional sources. The Journey of Mankind, for example, "a virtual global journey of modern man over the last 160,000 years" presented by the Bradshaw Foundation (Geneva, Switzerland), shows the interaction of human migration and climate (www.bradshawfoundation.com/journey/). This model is based on the work of Stephen Oppenheimer, a member of Green College at Oxford University, UK, and a fierce supporter of the so-called 'one-source theory'—the hypothesis that modern humans emerged from East Africa in a single migratory wave.

It is quite likely that the study of the genetic diversity of parasites and pathogens will provide new insights into human migration patterns if the results are integrated with other data on our history and if the limitations of this approach are carefully considered (see sidebar). "I think you'll need to look at every microbe on a case-by-case basis," Holmes commented. In addition to answering the question of where we come from and how we got here, knowledge of the evolution and diversity of pathogens might also help us to understand how virulence emerged and varies between different viral, fungal and bacterial strains. Given that diseases have always been closely linked with human movements and evolution, this research might open up new opportunities to develop therapies and preventive strategies against many infectious diseases that afflict specific human populations.

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A question of faith

Exploiting the placebo effect depends on both the susceptibility of the patient to suggestion and the ability of the doctor to instil trust

In the same way that physicians have generally had a rather ambivalent attitude towards the relationship between mind and healing, the placebo effect has had a long—and often troubled—relationship with conventional medicine. One of the first to describe the power of ineffective medicine was the French philosopher and essayist Michel de Montaigne, who wrote in 1572 "there are men on whom the mere sight of medicine is operative." In fact, in those days, nearly all medicines relied on either some form of placebo effect, or the natural ability of the patients to recover from an illness whether they were treated or not.

Despite being so well-known, it was not until the mid-twentieth century that clinical medicine took notice of the placebo effect. The term itself is often attributed—mistakenly—to the American pharmacologist and anaesthetist Henry Beecher, who had observed how the psychological state of men wounded in the Second World War seemed to influence their perception of pain. In 1955,

Beecher wrote, "three-quarters of badly wounded men, although they have received no morphine for hours [...] have so little pain that they do not want pain relief medication, even though the questions raised remind them that such is available for the asking" (Beecher, 1955). In fact, the term had been used more than three decades earlier in *The Lancet* (Graves, 1920).

Beecher's observations eventually led him to insist that clinical trials of new drugs could only yield reliable results if conducted in a double-blind fashion—that is, with a control group who were given a placebo without the knowledge of either the patients or the clinicians who administered the drug. Since then, there has been a tendency to regard the placebo effect as background 'noise' that must be subtracted from the results of a trial, rather than as a positive effect that could be exploited clinically.

In general practice, however, doctors widely prescribe placebos and are quite happy to acknowledge this anonymously. In 2004, a study of 89 Israeli physicians found