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From evolutionary advantage to disease agents: forensic re-evaluation of host-microbe interactions and pathogenicity

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Abstract

As the “human microbiome era” continues, there is an increasing awareness of our resident microbiota and its indispensable role in our increased fitness as holobionts. However, the host-microbe relationship is not so clearly defined for some human symbionts. Here we discuss examples of “accidental pathogens”, meaning previously non-pathogenic and/or environmental microbes thought to have inadvertently experienced an evolutionary shift towards pathogenicity. For instance, symbionts such as *Helicobacter pylori* and JC Polyomavirus have been shown to accompany humans since prehistoric times and are still abundant in extant populations as part of the microbiome. And yet, the relationship between a subgroup of these microbes and their human hosts seems to have changed with time, and they have recently gained notoriety as gastrointestinal and neuropathogens, respectively. On the other hand, environmental microbes such as *Legionella* spp. have recently experienced a shift in host range and are now a major problem in industrialized countries as a result of artificial ecosystems. Other variables involved in this accidental phenomenon could be the apparent change or reduction in the diversity of human-associated microbiota because of modern medicine and lifestyles. All of this could result in an increased prevalence of “accidental pathogens” in the form of emerging pathogens.

Keywords

paleomicrobiology; pathogenicity; symbiosis

Potential pathogens or ancient symbionts?

In a world dominated by microbes, our ancestors evolved alongside an outstandingly diverse ancient **microbiota** (see Figure 1 and Figure Box 1). Symbiotic relationships between animals, microbes and viruses have been observed as far back in animal evolution history as when *Hydras* made their appearance; and these interactions currently span across all types of life forms (1–3). It's clear that these ancient relationships have shaped the evolution of both the host and symbionts, although the selective pressures governing these processes remain unclear (4–6).

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As **holobionts** (see Table 1), our associated microbiota allows us to broaden our intrinsic capabilities and increase our survival fitness (7). From our postnatal development (8, 9) to post-mortem decomposition (10, 11) the human body is thoroughly influenced by, and most times highly dependent on, its associated **microbiome** and **virome** (see Table 1). The beneficial effects a stable **microbiota** (see Table 1) has on host fitness has been extensively reviewed, as these microbes often protect from disease (12–14), aid in food digestion (15–17), increment nutrient absorption and vitamin production (18–21), propel the maturation of host immune systems (22–25) and organ development (26), among many other attributes. However, an individual's microbiota is dynamic, always adjusting in response to changes in the needs and lifestyle of the host environment through time (27–29). In this chapter we will discuss examples of environmental and nonpathogenic human-associated microbes believed to have "accidentally" undergone a shift in their life cycles towards pathogenicity as a response to changes induced by or within their human hosts.

a) Helicobacter pylori

Microbial communities within holobionts are not stagnant, and population shifts within these communities are common (35, 36). However, a portion of these changes in the microbiota (abrupt or not) could result in detrimental effects to the host when mutualisms go awry (37). *Helicobacter pylori* is suspected to be one such case. *H. pylori* normally colonizes humans during infancy, a colonization that persists throughout the individual's life (38). Parent-child transmission is the most common manner (39), although people inhabiting the same area may acquire the microbe horizontally as well (40). Most *H. pylori* infections are asymptomatic (41–43). Interestingly however, under circumstances that still remain unclear *H. pylori* infections may predispose the host to gastrointestinal problems including duodenal ulcers and increased acid production (44, 45). Moreover, in certain populations *H. pylori* has been linked to gastric carcinoma (41, 46); it is therefore currently characterized as a group I carcinogen by the International Agency for Research on Cancer (47). Molecular methods of detecting and differentiating *Helicobacter pylori* strains are usually based on PCR amplification of DNA encoding for *16srRNA, H.pylori*-specific surface proteins, pathogenicity (e.g., ureases *ureA* and *ureC*) and microsatellites in the genome (48, 49).

H. pylori has been intrinsically associated to the human gut since our ancestors first migrated from Africa approximately 60,000 years ago (50). It is even theorized that this bacterium has accompanied our species since well before that time. A strong phylogeographic association has always existed between *H. pylori* strains during their co-evolution alongside ancient and modern human populations (51, 52). In fact, modern *Helicobacter pylori* strains are currently grouped into various geographical populations as a function of global human demographics and migrations (52). This strong phylogenetic association is mainly due to the bacterium's high genetic diversity as well as its mode of transmission (53). The high genomic diversity observed among *H. pylori* strains is associated to the bacterium's elevated mutation and recombination rates compared to other bacteria (54–57).

As a result of these characteristics, *Helicobacter pylori* has long been a prime candidate to determine past migrations (58–63), possible admixture (64) and genetic ancestry (65, 66) of

current and ancient cultures. For instance, genetic admixture and past migrations are evident with the widespread presence of European *H. pylori* strains in North and South America, which are possibly remnants of massive colonial expansions, while in comparison rural Amerindian communities commonly harbor strains of Asian origins, products of pre-Columbian migrations to the New World (67–69). Moreover, *H. pylori* strains associated to African subpopulations (hspWAfrica and hpNEAfrica) are commonly found in the United States (70), and were most likely introduced during the slave trade era. The genetic ancestry of individuals can also be assessed via *H. pylori* genotyping. In these ways, the identification of *H. pylori* genotypes could help microbial forensics determine the cultural origins of a human specimen under study. For example, although modern Europeans are mostly colonized by recombinant hpEurope strains, a 5300-year-old Southern European mummy (named Ötzi the "Iceman") was found to exclusively harbor an ancestral *H. pylori* strain of Asian origin named hpAsia2 (Figure 2) (71). Lack of similarity to European strains indicates that ancestral strains such as the hpEurope group settled in the population a mere several thousand years ago. Lack of genome hybridization in this ancient strain also suggested ancestral African strains (such as hpAfrica) had not been largely introduced into these European populations at that time.

If *Helicobacter pylori* have accompanied us throughout human history, has this relationship changed through time? It is unknown whether *H. pylori* infections in our ancestors were problematic. However, the asymptomatic presence of ancestral Asian strains in the guts of rural Amerindian communities in the Amazon and Peru (62, 65) suggests a previous, mutually-tolerated, or even possibly beneficial, relationship with Asian cultures that migrated to the area in prehistoric times (72–74). Nevertheless, this poses the question why the current shift to a more pathogenic dynamic with its host? As rural populations have been associated to higher microbial diversity (75–77), one possibility could be linked to the evident loss of the microbial diversity associated to our human ancestors as a result of modernization, cosmopolitan diets and exposure to antimicrobials (Fig.2) (78–82). This is supported by studies showing that hyper acidity and/or medical treatments that reduce the diversity in the gut microbiota allow *H. pylori* to germinate and proliferate without competition, causing an array of chronic diseases such as gastric inflammation and peptic ulcer disease (44, 45, 83, 84). In addition, a decreased diversity in the individual human microbiome has been associated to higher probabilities of developing an array of diseases, including obesity and diabetes (17, 85, 86). However, only some *Helicobacter* strains carry virulence genes, commonly associated to the acquisition of pathogenicity islands, including disease-linked genes, such as *CagA* and *VacA* which encode for cytotoxins (55, 87–89).

c) JC virus

Human Polyomavirus JC virus is also believed to be an ancestral symbiont that has co-evolved with humans throughout history. Similar to *Helicobacter pylori*, primary JC Polyomavirus (JCV) infections normally occur during childhood and are persistent throughout the host's life (90). In humans and other animals, possible JCV reservoirs include the kidneys, bone marrow, lymphoid organs and brain tissue (91–96). JC viruses are secreted for long periods of time in the urine and feces of infected individuals (97–100). Because of this, JCV transmission is believed to occur via the fecal-oral route and exposure to sewage-

contaminated waters (101, 102). Parent-child or transmission between family members is also common (103, 104). For these reasons, Microbial Source Tracking (MST) methods previously proposed JCV as an indicator of human fecal contamination in water reservoirs (105, 106). However, the possibility of other routes of transmission still remains unclear, including trans-placental transmission during pregnancy (107). JCV strains do not seem to be commonly transmissible between populations with established infections (108), therefore individuals co-habiting the same region usually have latent infections of the same JCV genotypes, whereas foreigners currently living in the area usually harbor a different strain from the established population.

Currently, JCV genotypes infect over 80% of the human population worldwide (109). JCV is identified and genotyped by PCR amplification of its VP1 major capsid protein and T antigen genes (106, 110). The distribution of the distinct JCV genotypes across the globe usually correlates with the presence of particular human populations in each geographical region. In fact, the dominance of certain genotypes in distinct ethnic groups suggests an ancient association with these populations (111). Although its limitations as a human marker have been argued (112), its strong host co-demography has also resulted in JCV to be considered a convenient historical and forensic biomarker to elucidate migrations, genetic heritage and admixture in modern and ancient human populations (Figure 4) (113–115). For instance, the complex genetic history of African American communities in the United States is reflected with the presence of JCV genotypes associated to European (Types 1 and 4) and African regions (Type 3) in these individuals (116). Similarly, JCV genotypes present in native Japanese communities (CY and MY) are shared among subsequent Japanese generations, even those that migrated to other continents (104). Interestingly, the Japanese MY genotype has been detected among several South American Amerindian cultures (117) and was presumably introduced to the region by pre-Columbian ethnic groups from the Pacific. In addition, preliminary forensic studies in modern Puerto Ricans revealed the presence of seven JCV genotypes, originating from Spain, Africa and Asia (118). In this case, detected JCV genotypes also mirrored the known migration and genetic history of the population, as the Asian strain detected in these individuals was probably obtained from indigenous cultures migrating to the Caribbean in ancient times (119–122). Finally, JCV strains Type 2 and 7 (of Asian origin) were detected in rural communities located in the highlands of Papa New Guinea, whereas populations in Guam only had one Asian strain (123). This again associates to the settlement of different areas of the Pacific by people coming from various Asian continents.

Historically, JCV has received much attention in clinical settings due to its isolation from the brain of a patient with progressive multifocal leukoencephalopathy (124) and subsequent associations with severe brain tumors in immunosuppressed patients (125–130). However, in the immune-competent JCV infections are often asymptomatic (131–133). In addition, whole-genome phylogenetic analyses of JCV genotypes suggested JCV type 6 is an ancestral variant that colonized humans before their exodus from Africa, and has since diverged into two distinct evolutionary lineages, each with multiple subpopulations (134). This suggests that the relationship resulting of the co-evolution between our species and JCV was initially not meant to be detrimental to the host, but is being pushed towards a pathogenic cycle as a result of other unknown influences.

Environmental microbes turned human nightmares

a) Mycobacterium tuberculosis

Tuberculosis (TB) is a disease caused by a member of the *Mycobacterium* genus, an obligate pathogen that can infect any organ of the body but is usually associated to the highly oxygenated tissue (e.g., lungs). TB, particularly pulmonary tuberculosis, has been a global threat to both humans and animals for ages. It is currently present in a third of the world's population (136), in many cases as latent infections with multi-drug resistance (137). Currently, cases are commonly linked to diabetes, tobacco use, AIDS and/or deteriorated social conditions (138). Today, most TB cases are caused by *Mycobacterium tuberculosis* (Mtb), one of many *Mycobacterium* species forming the *Mycobacterium tuberculosis* complex of organisms (Mtb C), which also includes *Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microti,* and *Mycobacterium canetti*. (139). *Mycobacterium bovis* is also a causing agent of TB in humans and other organisms (Division of Tuberculosis Elimination 2014; Cosivi et al. 1999).

i. Past and current 'tuberculli'—MTb is usually identified by Restriction Fragment Length Polymorphism (RFLP) of its IS6110 insertion sequence (142). However, because of their essentially clonal genomes, Mtb strains are usually differentiated among each other via CRISPR spoligotyping (143) and profiles of mycobacterial interspersed repetitive units present in variable number tandem repeats (MIRU-VNTR) (144). Whole-genome sequencing is also used to identify Mtb strains. These techniques have proven essential to microbial forensics when tracking outbreak origins. For example, from May 2006 and December 2008, a tuberculosis outbreak was observed in a medium-size community in British Columbia, Canada; it affected 41 individuals with an incidence rate of 6.4 cases per 100,000 population (145). None were classified as foreign-born or human immunodeficiency virus-infected individuals. In this case, whole genome sequencing of 36 *M. tuberculosis* isolates found the strains were essentially identical, indicating a clonal outbreak (146). However, in-depth phylogenetic analyses showed the presence of two major genetic lineages, suggesting it was actually two clonal outbreaks that occurred simultaneously. Both lineages had been sporadically detected in the area since at least 2005. Epidemiological, social-network and molecular forensic analyses determined the Index patient was an adult with an asymptomatic pulmonary Mtb infection that had been untreated for 8 months.

In another study, genotyping of Mtb strains involved in a 4-fold increase in tuberculosis cases in Indiana from 1996-1999 determined the DNA fingerprints were highly similar between the strains detected throughout the outbreak (147). Findings suggested inadequate handling of the latent risks driving the transmission of the pathogen was responsible for the spread throughout the community. Thus, spread was mainly due to lack of identification of infected individuals and persons at highest risk of subsequent infection. Another example of contact tracing in tuberculosis outbreaks was conducted in a high school in Alabama, where a 13 year-old was found to be the source patient that infected 30 percent of students and staff attending the same school (148). Transmission via exposure of the same classroom and

school as source patient resulted in around 200 new cases of tuberculin reactivity and 6 cases of active tuberculosis.

Currently, phylogenetic studies have classified 6 main lineages grouping strains from (i) Europe and Americas, (ii) East Asia, (iii) India and East Africa, (iv) West Africa, (v) the Indian Ocean and (vi) the Philippines as defined major clusters (149). The dispersal of each main lineage seems to correspond to the migration patterns of human populations from that geographic area (150). This may explain why pulmonary and spinal TB's notoriety were so well documented across many ancient civilizations. In ancient Greece it was known as '*phthisis*' (151, 152); it was also recorded in ancient India, Asia and Turkey (153, 154). Tuberculosis-associated "hunchbacks" were also represented throughout Egyptian art (155). Although thousands of years have passed since the first recorded accounts of the disease, ancestral and current strains remain very similar. The oldest human-associated Mtb genetically confirmed and characterized to date is from a Neolithic population in the Mediterranean (156). This 9,000 year-old Mtb showed high genome similarity to extant strains. In fact, forensic studies have identified an ancient composite genome present in modern variants (157). This composite genome is potentially the product of a bottleneck event or high rates of intraspecies horizontal transfer in MtbC early history, and it is remarkably still detectable even despite recent genome restructuring. Moreover, ancient and extant Mtb strains share common deletion events of *TbD1* in their genome, again suggesting an ancient common ancestor (158).

ii. Origins of the 'White Plague'—Previous hypotheses suggested that TB epidemics began in 18th century Europe and spread to Africa and the Americas (159, 160). However, this speculation was mainly due to forensic studies not showing confirmed tuberculosis cases in ancient Africa and the New World, especially compared to the high number of Mtb-infected specimens in pre-Columbian Europe. Nevertheless, studies detected MtbC in pre-Columbian individuals from Peru (161). Although some consider the sample dates controversial (162, 163), these findings advocate the presence of the tuberculosis pathogen prior to European contact in South America.

Current data indicates MtbC originated in East Africa, where an ancestral precursor of MTB infected hominids around 3 million years ago (164). This hypothesis is known as "*Out-Of-And-Back-To-Africa*" for human-associated MtbC, in which this precursor strain would have accompanied and evolved alongside mankind as we migrated from Africa. As of yet, this has not been detected in African hominid skeletons from that time period (although several Egyptian mummies have been confirmed as MtbC-positive (155, 165, 166)). However, the high genomic similarity between ancient and extant Mtb strains clearly supports the hypothesis of a long-term co-evolution with its human hosts. In addition, the geographic location of modern Mtb strains also suggest human MtbC originated in Africa, as this is the only place where all six human MtbC major lineages have been detected (149).

One question remains however: was MtbC originally a human pathogen? Some have argued that MtbC was zoonotic long before its contact with humans; acquiring its infectivity to humans posteriorly (159, 168). Interestingly, forensic studies showed the MtbC strain detected in pre-Columbian Peruvians had higher similarity to strains infecting seals than

human-associated modern strains. On the other hand, recent data suggests that MtbC could have initially been a free-living microbe that slowly acquired strategies allowing it to persist in humans and animals (169). This idea is supported by the vast amount of virulence factors detected in pristine environments (Søborg et al. 2013; Gonzalez et al in preparation) as well as the diversity of *Mycobacterium* species commonly observed in the environment, especially in water and soil (for a review on this subject please see: Hruska & Kaevska 2012). Furthermore, *M. bovis* and *M. tuberculosis* are capable of long-term survival in soil and also maintain their virulence throughout soil exposure (172). But if human MtbC was initially an environmental microbe, how did it end up infecting humans? One hypothesis for transmission is known as the Pleistocene campfire theory, and proposes that MtbC was acquired by ancient humans as a result of social interactions around wood fires (173). This possibility has a certain appeal, as the inhalation of carcinogens present in wood smoke lowers our immunological defenses (174, 175) and could have thus paved way for those first MtbC infections in humans. Notably, despite our best intentions at deciphering the history of TB in humans one thing is clear: further studies on ancient and extant strains are needed to effectively elucidate the evolution and epidemiology of this ancient human pathogen.

b) Toxoplasma gondii

Toxoplasma gondii is the protozoan responsible for latent toxoplasmosis, which infects over 20% of the population in the United States of America. Although it can infect many warm-blooded vertebrates as intermediate hosts, only infecting cells lining the gastrointestinal tract of a felid allows the pathogen to mature and reproduce (176). Humans are usually infected by *T. gondii* via exposure to feces-contaminated soil, congenital transmission (177) or consumption of uncooked contaminated meat (178). Latent toxoplasmosis is usually considered asymptomatic in the immunocompetent. However, *T. gondii* infections notoriously alter host brain and behavior to its own advantage (179). For example, in rats *T. gondii* causes a lack of fear towards cats (180, 181) and even causes rodents to be sexually aroused by their feline predators (182). This is presumably to increase the chances of the intermediate host to be eaten by the parasite's final host, thus allowing the brain *T. gondii* to complete its life cycle. In other animals, such as goats, toxoplasmosis has been linked to abortions and perinatal death (183, 184). In humans, latent toxoplasmosis has been linked to an apparent increase in host neuroticism (185) and suicide attempts (186), among other behavioral manipulations (187).

Toxoplasmosis is usually serologically diagnosed using IgG, IgM, IgA, IgE antibody titers specific to *T. gondii* (188). Agglutination tests are also common. Molecular characterization of *T. gondii* is done by PCR of its B1 gene, and strain genotyping is conducted based on microsatellite markers such as *TUB2, W35, TgMA, B18* and *B17,* among others (189). Initial studies on *T. gondii* populations suggested they were clonal from one ancestor. On the other hand, in-depth analyses discovered genetic diversity between strains (190, 191) and phylogenetic analyses suggest this pathogen's history includes both clonal and sexual strain propagation (191). Although this pathogen is highly prevalent in many communities, documented, in-depth investigations of toxoplasmosis outbreaks are not common. There are some exceptions however. For instance, source tracing of a toxoplasmosis outbreak in British Columbia, Canada determined transmission of the disease occurred via a

contaminated municipal drinking water tank (192). A village in Patam, Burma, suffered a toxoplasmosis outbreak in late 2003, which resulted in the deaths of an adult, a neonate and a fetus. Although no epidemiological sources were identified, studies determined that clonal isolates from one atypical *T. gondii* strain were responsible for the outbreak (193). Similarly, an outbreak that resulted in 155 reported cases of toxoplasma in Brazil was also studied (194). In this case, unfiltered, municipally treated water was determined as the epidemiological source of infection. All strains implicated in the outbreak expressed SAG-2 parasite-specific antigens and were classified as the same genotype (type I). However, there was insufficient genomic information to determine if this was a clonal outbreak. Outbreaks have also been observed in Hawaiian monk seals after exposure to marine water contaminated with feline feces (195).

c) Legionella pneumophila

Legionella pneumophila is a facultative intracellular parasite infecting protozoa in freshwater and soil (196, 197) (for a review see, Bitar et al. 2004). In recent years however, *L. pneumophila* has been prevalent in man-made water systems, including those found in cars (199), water distribution systems and buildings (Center for Disease Control and Prevention, 2016). In these reservoirs, *L. pneumophila* infects amoeba that upon inhalation act as a “Trojan horse” in humans (200, 201). Due to constant exposure to these water systems in infected infrastructures, cases of Legionellosis are becoming common in human populations (202). For example, in 1994 fifty cases of Legionnaires' disease were reported among passengers of a cruise ship; cases were posteriorly linked to a contaminated whirlpool spa on the ship (203). In 2005, an outbreak was observed in an extended care facility in Ontario, Canada, resulting in 23 deaths (204). The source of the outbreak was identified as a contaminated air conditioning cooling tower. Genetic assays determined high similarity between clinical strains associated to the outbreak and environmental strains from the outbreak site.

In a way, the adaptations *L. pneumophila* obtained during its co-evolution with its protozoan hosts accidentally played a key role in its recent host switch towards humans (205). This is because once it is inside the human body, *L. pneumophila* is now capable of infecting its host's macrophages (206, 207). This is mainly due to similarities between the microbe's environmental amoeba host and human macrophages (208, 209). In addition, the same genes are expressed by *L. pneumophila* during the infection of both host cells (210). Also, *L. pneumophila* protects itself by hijacking and replicating in membrane-bound compartments, vesicles or “vacuoles” within both host cells (for a review, see Isberg et al. 2009). Given the known evidence of its epidemiology, it is highly likely that this bacterium is actually an “accidental human pathogen” (212). Thus, *Legionella pneumophila* is an excellent example of how our industrialized environment is capable of providing the stage for a previously environmental, non-pathogenic microbe to shift towards humans as hosts.

Concluding remarks

We are slowly beginning to understand the complexity driving host-microbe interactions and the shifts in the microbiome and virome associated to changes in the host environment.

Studies in extant individuals indicate that several current diseases are triggered by the use of antimicrobials, eliminating microbial competition and allowing for potentially pathogenic microbes to increase in numbers within the host. This is especially true for microbes possessing virulence factors that depend on quorum sensing molecules to be expressed (214, 215). As holobionts, we seem to have eliminated a portion of our associated microbes across evolutionary time. Indeed, it is possible that the “opportunistic pathogens” discussed in this section could have been a part of a previously beneficial symbioses with humans that went awry, thus rendering these microbes “accidental pathogens”. On the other hand, some of the beneficial symbiotic relationships between ancient humans and microbes still persist as such to this day. Many of these microbes have co-diversified alongside their human host populations since then. It remains unclear, however, which selective pressures have shaped these relationships. Such “micro-evolution” stories now present high phylogeographical correlations to specific human populations.

Many aspects surrounding host-microbe interactions remain unclear; but many unknowns are being explained using molecular forensics for the first time. One thing is clear however; the mutualism-parasitism continuum that governs a microbe-host relationship through time is now seen as something complex and dynamic, where a microbe can change its effect and relationship with the host in response to environmental stimuli. This in turn results in a microbial version of the Red Queen effect (216), a constant competition between the members of this symbiosis, where the host tries to control symbiont populations and the symbiont evolves ways of circumventing these measures (217). Over time, we seem to have lost many of these ancient symbioses with our microbial partners. This could be due to migrations, shifts in diets or lifestyle changes, resulting in a less diverse gut microbiome in modern humans. Symbionts can indirectly protect the host from pathogen infection and colonization through constant activation of the immune system and alteration of cell morphology. Cross-protection has also been frequently observed as a result of particular virus infections. For instance, herpes viruses, which persist as latent infections ubiquitous in modern humans, seem to give the host immunity towards the pathogens *Listeria monocytogenes* and *Yersinia pestis* (218). It is apparent that the disappearance of these ancestral human microbiota may have helped shift the ‘mutualism-parasitism continuum’, inducing in the human host a higher predisposal to several of today's widespread diseases. Our ability to carry out molecular microbial forensic studies is elucidating many of the previously undetermined interactions between humans and emerging accidental pathogens.

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Fig. 1. *Endosymbiosis: Homage to Lynn Margulis*. Artist: Shoshanah Dubiner

This painting illustrates a portion of the incredible microbial complexity that existed on this planet when animals evolved, and thus the microbial soup in which all organisms developed. (Image courtesy of the artist. Image credits: *Endosymbiosis: Homage to Lynn Margulis*, Shoshanah Dubiner, 2012. www.cybermuse.com.)

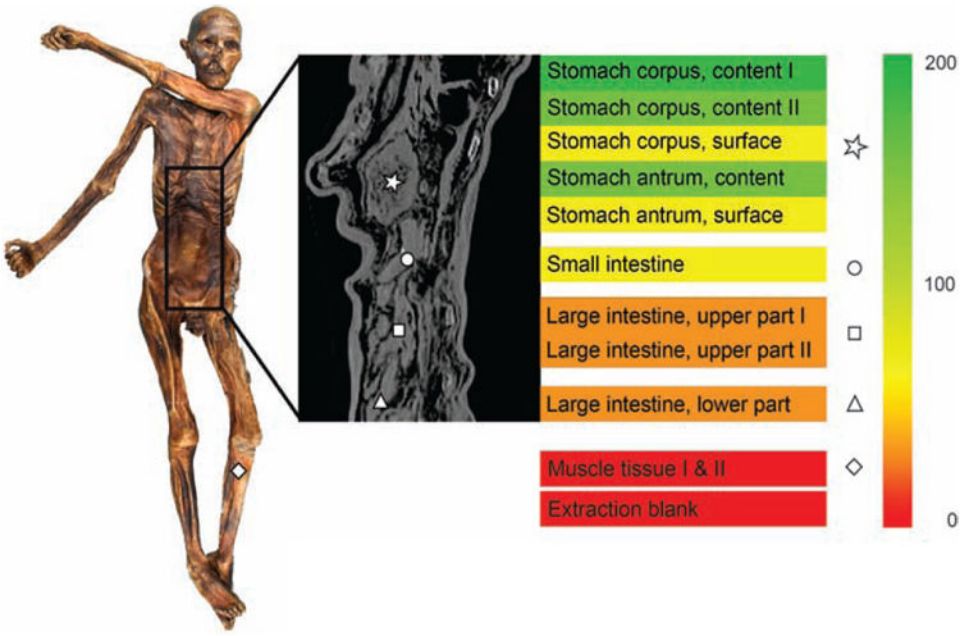

Figure 2. *Helicobacter pylori* in Ötzi the 5300 year-old Iceman

Reads specific to *Helicobacter pylori* were detected via metagenomic analysis of DNA extracted from different regions of the gastrointestinal tract of Ötzi, the Tyrolean Iceman. In this figure, the area where the muscle control sample was obtained is highlighted as a diamond (picture on the left), whereas the gastrointestinal sampling sites are marked in the radiographic image using the following legend: asterisk, stomach content; circle, small intestine; square, upper large intestine; triangle, lower large intestine. The number of *Helicobacter*-specific reads per million metagenomic reads is indicated by the colored gradient bar on the right. (Credits: Maixner et al, Science, 2016. Fig.1 (71)).

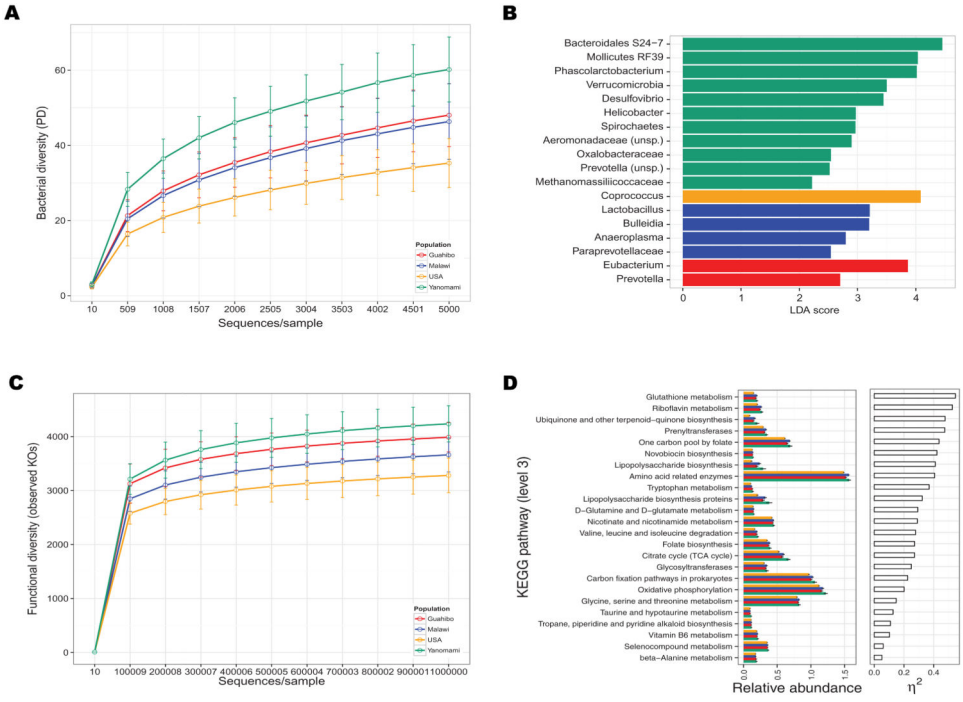

Figure 3. A lower fecal microbiome diversity associated to individuals from industrialized cultures

Dominguez-Bello and her team compared the bacterial diversity in feces from cultures with hunter-gatherer lifestyles compared to progressively more industrialized cultures. A) Phylogenetic diversity in feces from Yanomami and Guahibo Amerindians, Malawians and U.S. individuals. A higher bacterial diversity was detected in feces from the Yanomami, an isolated, rural indigenous culture inhabiting the Amazon. In comparison, a slightly decreased fecal diversity was found in Guahibo Amerindians. However, a major decrease was detected in the diversity of the fecal microbiota in U.S. subjects. A pronounced decrease was also detected in the functional profiles of fecal microbiomes from U.S. subjects compared to cultures with more traditional lifestyles (figure not shown). (Credits: Clemente, et al. Sci Adv. 2015. Fig.1A.).

B) Key differential bacterial groups between fecal microbiomes from Yanomami and Guahibo Amerindians, Malawians and U.S. subjects. (Credits: Clemente, et al. Sci Adv. 2015. Fig.1C).

C) Functional diversity in feces from Yanomami and Guahibo Amerindians, Malawians and U.S. individuals. As expected, a higher overall functional diver(Credits: Clemente, et al. Sci Adv. 2015. Fig.2A.).

D) Comparison of major metabolic pathways detected in fecal microbiomes from Yanomami and Guahibo Amerindians, Malawians and U.S. subjects. (Credits: Clemente, et al. Sci Adv. 2015. Fig.2C).

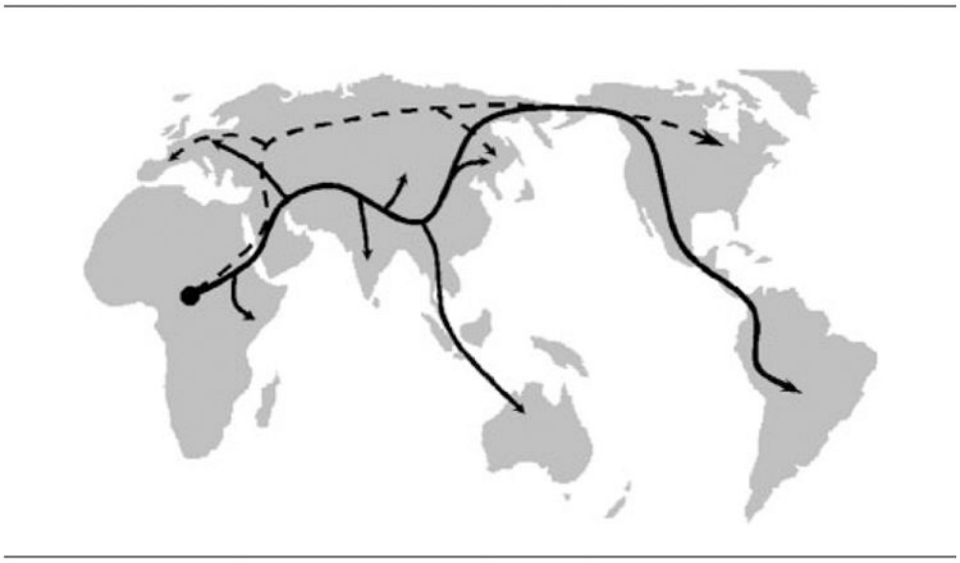

Figure 4. Forensic studies with JCV DNA suggest the expansion of *Homo sapiens* from prehistoric Africa occurred as a two-migrations model

As Pavesi shows in his model, two out-of-Africa migrations were suggested by currently characterized JCV subtypes. The first migration, represented with a solid line, is compatible with that previously suggested by human genes. The second migration, traced with a dashed line, indicates an additional route of expansion suggested by JCV, but that is undetectable using only human genes. Credits: Pavesi, J Virol, 2005 (135).

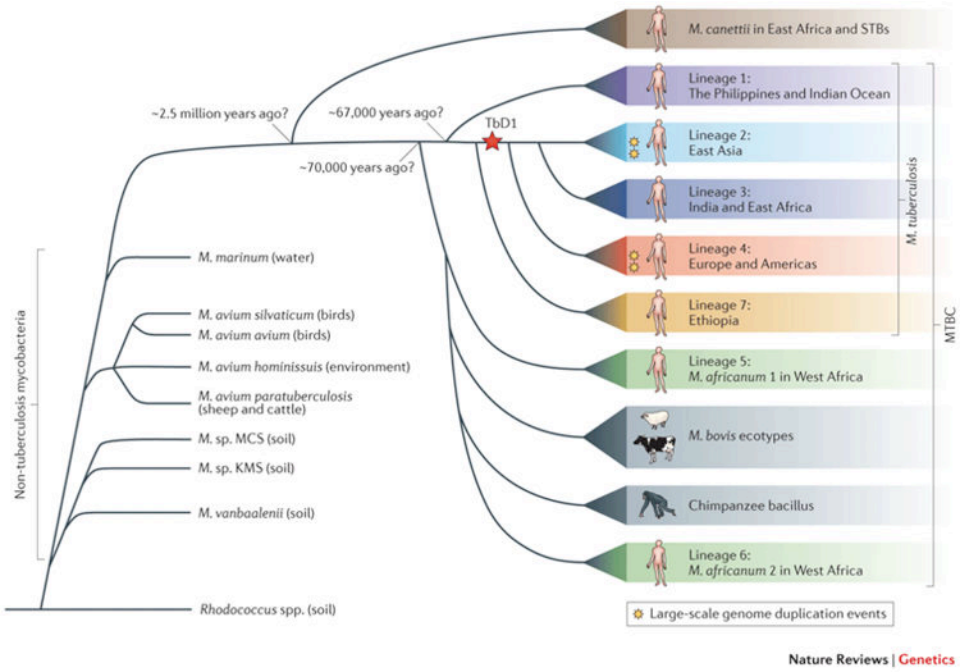

Figure 5. Origin of human-associated *Mycobacterium tuberculosis*

Although its exact ancestral history remains unresolved, recent studies clearly suggest *Mycobacterium tuberculosis* was associated to humans previous to their expansion from prehistoric Africa. However, *M. tuberculosis* is believed to have been an environmental microbe long before its association to ancient humans. This figure was taken from Galagan, Nature Reviews Genetics, 2014, and depicts a summary of the conclusions implied by current phylogenetic literature on the evolution of *M. tuberculosis* and other members of the *Mycobacterium tuberculosis* complex (167).

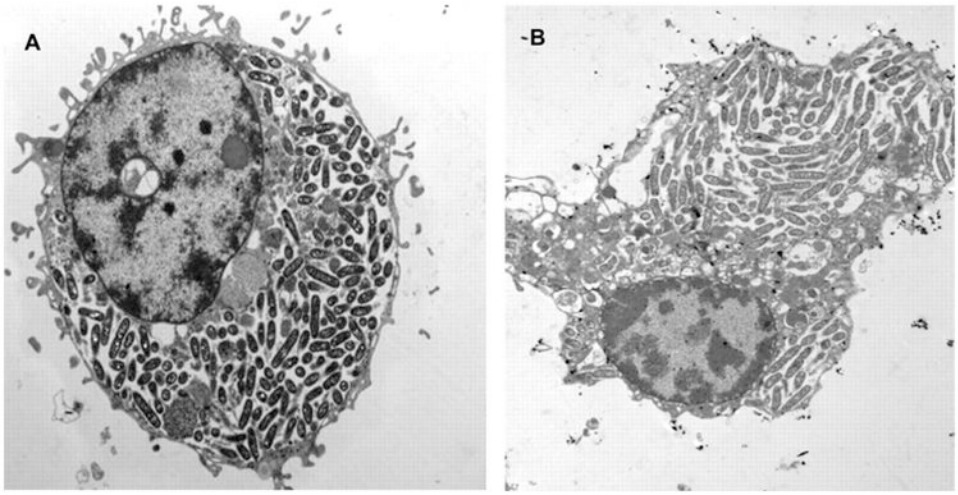

Figure 6. ***Legionella pneumophila*** **infections in environmental amoebae and human macrophages**
Electron micrographs of U937 macrophages (A) and A. polyphaga (B) infected by L. pneumophila (strain AA100) at 24 h. Image taken from Molmeret et al, AEM, 2005 (213).

Table 1

Definition of terms used from microbiology and virology

Term	Definition	References
Microbiota	Microorganisms associated to a habitat or host.	(30, 31)
Holobiont	The total collection of life present in an organism, including the host and its associated microorganisms, viruses and phages.	(7)
Microbiome	The collection of microbial genomes present in a habitat or host	(32, 33)
Virome	The collection of virus and phage genomes in a habitat or host	(34)