1) Ancient DNA of both pathogens and humans is expected to undergo damage over time, resulting in characteristic nucleotide misinformation patterns. However, when scientists studied DNA samples of *Mycobacterium leprae* taken from medieval skeletal remains from Denmark, Sweden, and the United Kingdom, they found that as compared with human mitochondrial DNA from the skeleton, the pathogen DNA had undergone very little damage. To account for this, scientists hypothesized that this type of DNA preservation pattern may be because the mycobacterial cells are surrounded by a thick, hydrophobic layer of mycolic acids that account for around 40% of the cell biomass. The mycobacterial cells which showed the least DNA damage had exceptionally high levels of mycolic acids around the cell. Hence, it was concluded that the lipid-rich cell wall of *Mycobacterium leprae* protected the mycobacterial DNA from hydrolytic damage resulting in long DNA fragment lengths and reduced nucleotide misincorporation patterns.

2) Figure 3B represents a Bayesian phylogenetic tree which includes all ancient *Mycobacterium leprae* strains along with their radio-carbon dates in comparison with the newly identified and studied strains from skeletal remains. The figure represents the divergence times for *M. leprae* strains denoted by rate variations among the lineages. The x-axis denotes divergence time intervals ranging from 1000 BCE (1000 years before the current era) at the extreme left to 2000 CE (2000 years into the current era) at the extreme right. Based on the SNP (Single Nucleotide Polymorphism) typing scheme, most mycobacterial genomes were classified into four branches, except for two which were classified as Branch 0 and formed the deepest lineages with their lineage lines going all the way to the right. The tips of the branches correspond to the radiocarbon dates of ancient samples and isolation dates for modern samples. The divergence time on the x-axis corresponding to the tips of the branches denote the point in time when the particular strain had diverged from the mother strain. Based on calculations by the software BEAST, the divergence times for all *M. leprae* strains have been estimated to be around 2871

years ago which is consistent with the oldest known and accepted evidence of leprosy in India at around 2000 (BCE).

3) In the 16th century, Europe saw a sudden decline in the prevalence of leprosy in the region; however, its prevalence remained high in the other regions around the world. This decline was definitely not due to a loss of virulence as previously hypothesized because modern *M. leprae* strains show a considerable homogeneity with ancient strains indicating similarity in the levels of pathogenecity and virulence. Hence, in order to explain its sudden decline, it has been proposed that several extraneous factors might have a role to play in the disappearance of leprosy from the region. These factors may include rise and prevalence of other infectious diseases such as plague and tuberculosis, alterations in host immunity, and improved social conditions leading to widespread action taken to address the incidences of leprosy in Europe.