The origins and evolution of *Mycobacterium leprae*

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Background

Human population growth has led to a drastic reduction in wildlife habitats. It is important to understand which pathogens carried by other animals, especially primates, can be transmitted to humans living in close proximity. Additionally, humans may also transmit pathogens to primates, endangering their populations and thwarting conservation efforts[1].

Leprosy is an ancient disease, primarily caused by *Mycobacterium leprae*. Natural infections of *M. leprae* have been reported in armadillos, chimpanzees, sooty mangabeys, and cynomolgus macaques[2-10]. In mice, rats, and cats, leprosy is caused by *M. lepraemurium*[11]. The clinical and histopathological manifestations of *M. lepraemurium* infection are similar to those of *M. leprae* (Figure 1).

M. leprae is an obligate parasite with a severely reduced genome[12]. This genome reduction is hypothesized to have occurred 12-20 million years ago[13], long before the origin of anatomically modern humans. Research suggests that there is less diversity among human strains of *M. leprae* and that this pathogen originated in humans about 3500 years ago[14]. These results can be explained by the pathogen having undergone either a bottleneck event or a host jump from another species to humans. To elucidate the evolutionary history of leprosy, we aim to investigate strains of *M. leprae* and other potentially closely-related mycobacteria isolated from non-human primates as well as other animals.

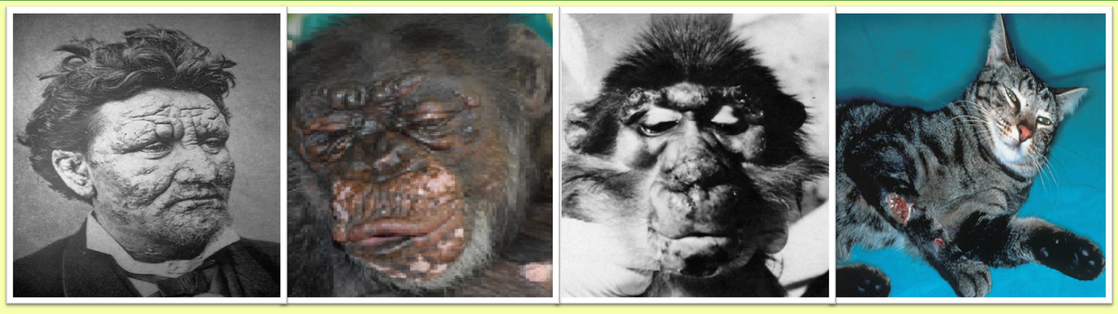

Figure 1: The first three panels depict characteristic symptoms of leprosy in a human[23], chimpanzee[7], and sooty mangabey[9] infected with *M. leprae.* The fourth panel depicts localized leprosy lesions on the forelimb of a cat with *M. lepraemurium* infection[15].

Objectives

- To use genome data from a strain of *M. leprae* isolated from a West African sooty mangabey (*Cercocebus atys*) and identify its phylogenetic relationship with *M. leprae* strains isolated from humans.
- To carry out whole-genome sequencing of *M. lepraemurium* strain Hawaii and determine its phylogenetic relationship with *M. leprae.*

Methods

- *M. leprae* mangabey strain whole-genome sequencing[24].
- *M. lepraemurium* strain Hawaii DNA extraction, library preparation[22], and whole-genome sequencing using Illumina MiSeq technology.
- Quality-filtering of sequence reads using a bioinformatics pipeline[16,17].
- Mapping assembly of filtered reads to appropriate reference genomes using Geneious[18].
- Multiple whole-genome alignments using MAUVE[19].
- Phylogenetic analyses using MEGA6[20].

Results

Analysis of the *M. leprae* mangabey strain

- Reads mapped to reference genome (*M. leprae* strain TN) resulted in a consensus sequence of 3,268,203 base pairs. Mean coverage obtained was 53X.
- The *M. leprae* mangabey strain belongs to subtype 4N based upon characteristic single-nucleotide polymorphisms[21] and is grouped along with other Branch 4 strains (Figure 2).

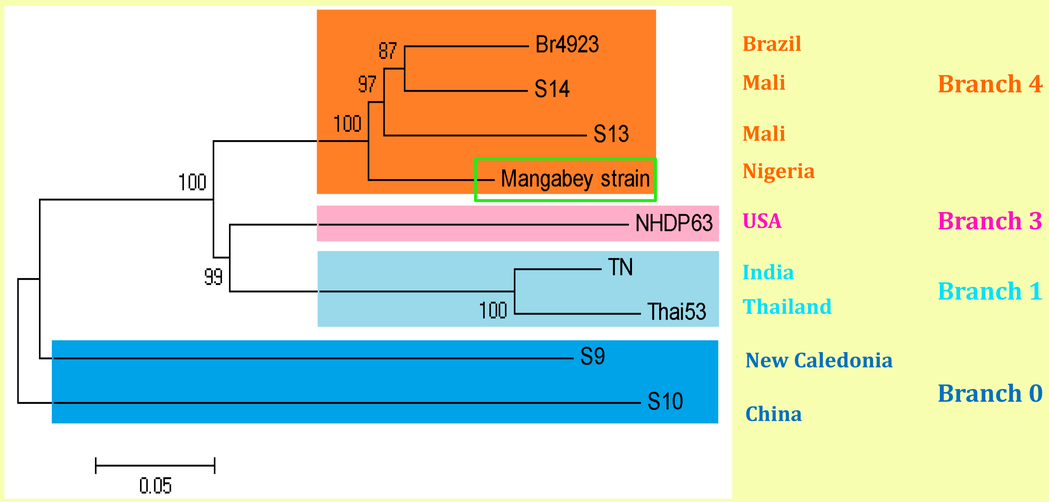

Figure 2: Maximum Likelihood tree based on 497 variable nucleotide positions. All positions containing gaps and missing data were eliminated. The tree was based on the HKY model, determined to be the best model of evolution from the AIC values generated using MEGA's model test. Bootstrap support was generated using 1000 replicates and the percentage of trees in which the associated taxa clustered together is shown next to the branches. Geographic origin is given adjacent to the name of the strain. The branches are represented by different colors.

Analysis of the *M. lepraemurium* strain

- 96.5% of reads mapped to the reference genome (*M. avium* strain 104) and resulted in a consensus sequence of 4,196,484 base pairs. Mean coverage obtained was 22X, with a maximum coverage of 238X.
- *M. lepraemurium* is grouped with members of the *M. avium* complex and is not closely related to *M. leprae* (Figure 3).

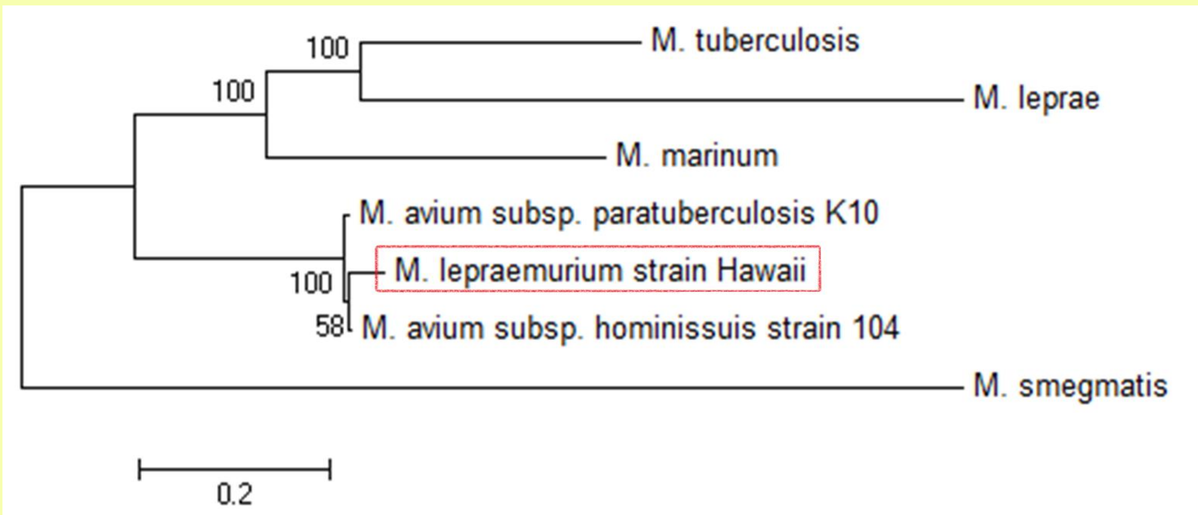

Figure 3: Neighbor-Joining tree based on 6857 variable nucleotide positions. All positions containing gaps and missing data were eliminated. The tree was based on the GTR model, determined to be the best model from the AIC values generated using MEGA's model test. Bootstrap support was generated using 1000 replicates and the percentage of trees in which the associated taxa clustered together is shown next to the branches.

Discussion

- Analyses of the *M. leprae* strain isolated from the sooty mangabey show that it is similar to human *M. leprae* Branch 4 strains. Branch 4 strains are found primarily in West Africa, and in the Caribbean where they were introduced due to the slave trade[21]. The mangabey was imported from Nigeria to the US for research purposes and never came into contact with a known source of leprosy[8]. Therefore, the mangabey probably acquired leprosy from a human, in West Africa, prior to being shipped to the US.
- Preliminary analyses carried out on the *M. lepraemurium* genome sequence suggest that it is not closely related to *M. leprae* and it belongs to the *M. avium* complex.

Future Work

Future work will involve whole-genome sequencing and analyzing *M. leprae* strains isolated from a chimpanzee[7] and a cynomolgus macaque[10] with naturally-acquired leprosy.

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