

The Emerging Phenotype of Ancestral Tuberculosis

PROF DR BOUKE DE JONG
INSTITUTE OF TROPICAL MEDICINE, ANTWERP

TB SYMPOSIUM-MARCH 2023



LES BACILLES TUBERCULEUX DE TYPE AFRICAIN

1968

NOTE PRÉLIMINAIRE

Dakar: *M. africanum* West-African 2
MTBc Lineage 6

par

M^{me} M. CASTETS (1), H. BOISVERT (2), M^{me} F. GRUMBACH (3),
M. BRUNEL (4) et N. RIST (3).

1970

Further studies on African strains
of *Mycobacterium tuberculosis*.

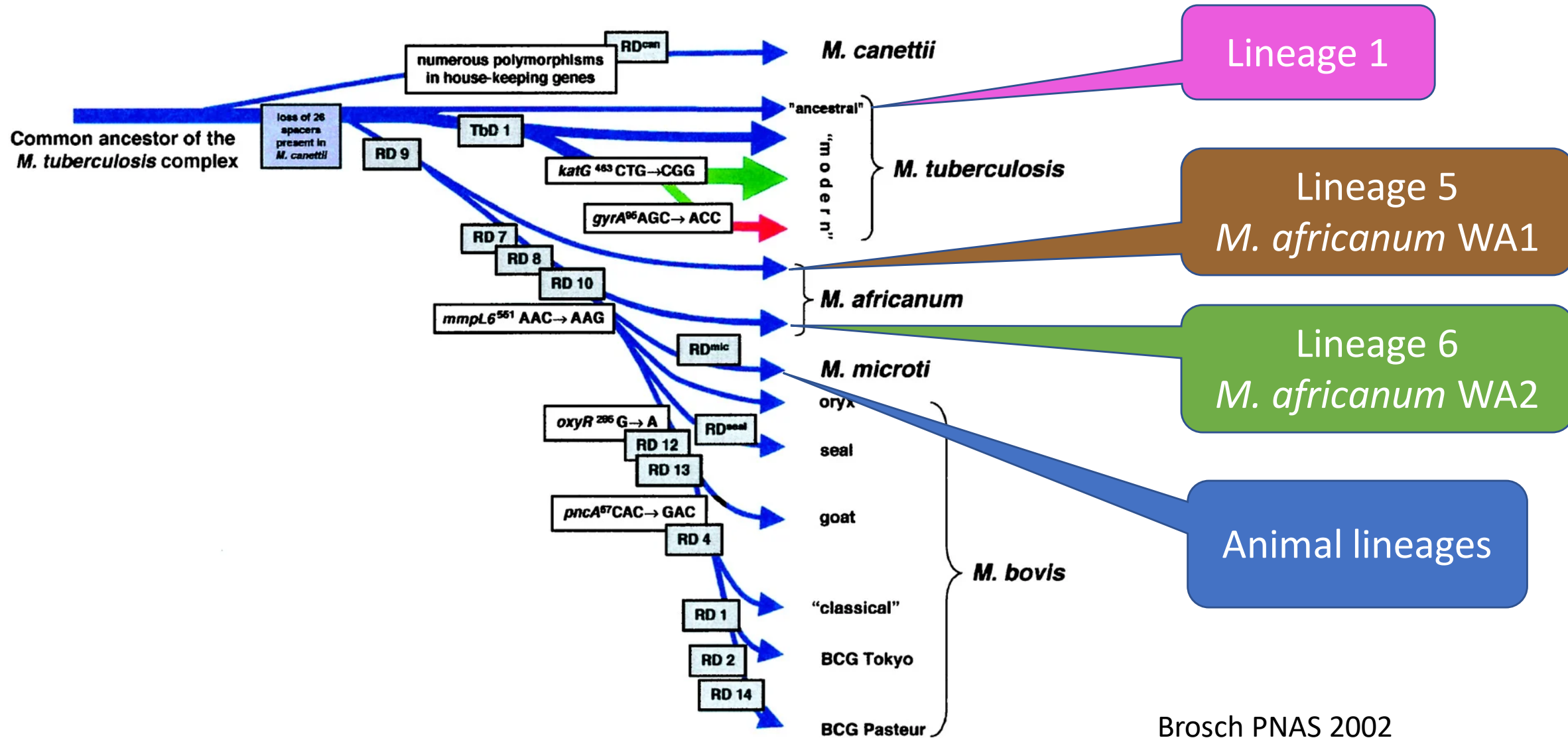
Comparison with *M. bovis* and *M. microti*

BY

S. R. PATTYN, F. PORTAELS, L. SPANOGHE, and J. MAGOS

When viewed from the standpoint of numerical taxonomy, these strains are highly different from *M. microti* and *M. bovis* but closely related with *M. tuberculosis*, the differences with *M. tuberculosis* are not greater than the differences among each other so that to consider them as a different species is not justified.

2002: modern lineages lack TbD1

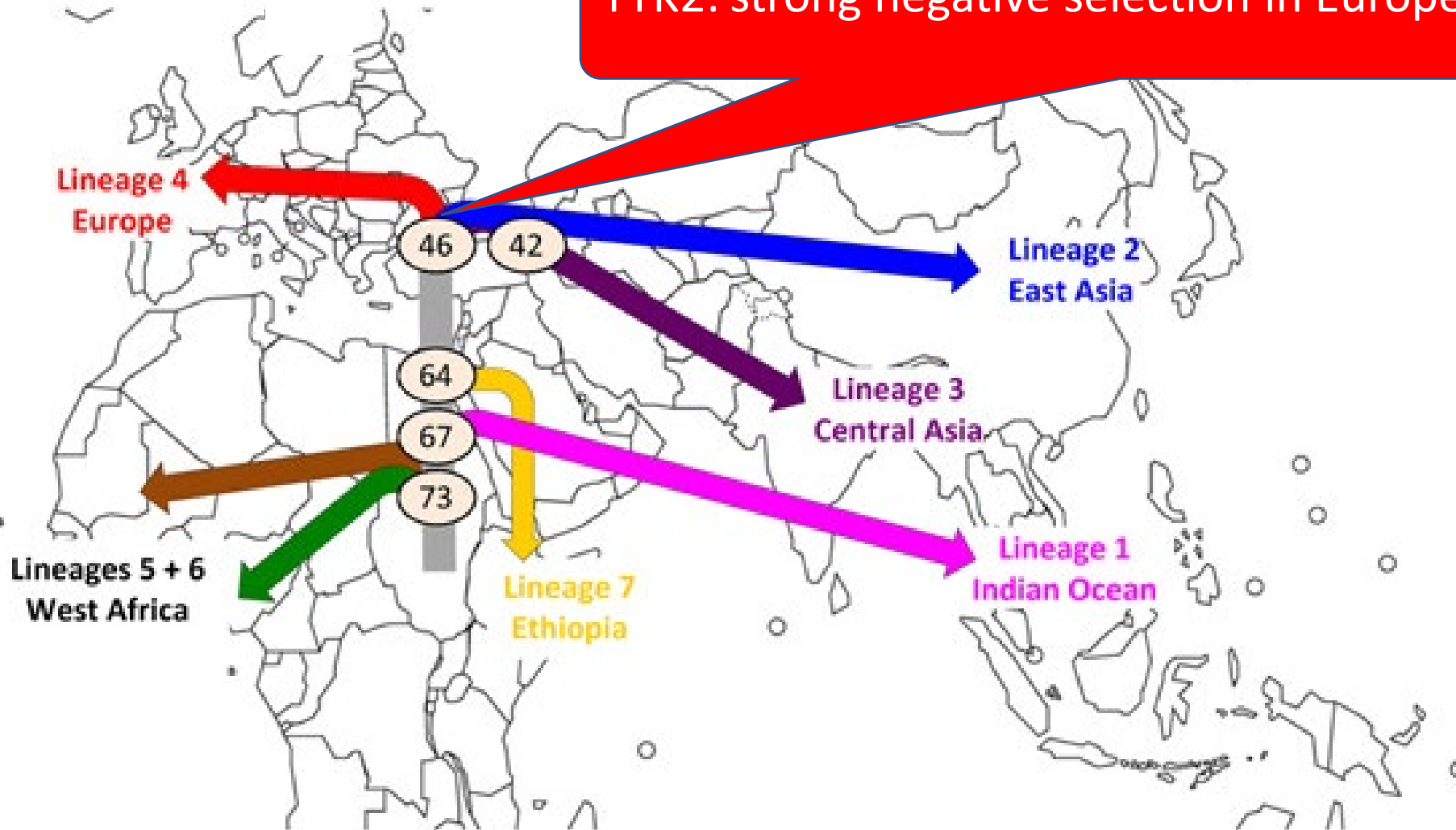


Brosch PNAS 2002

MTBc: African origin, age unsure

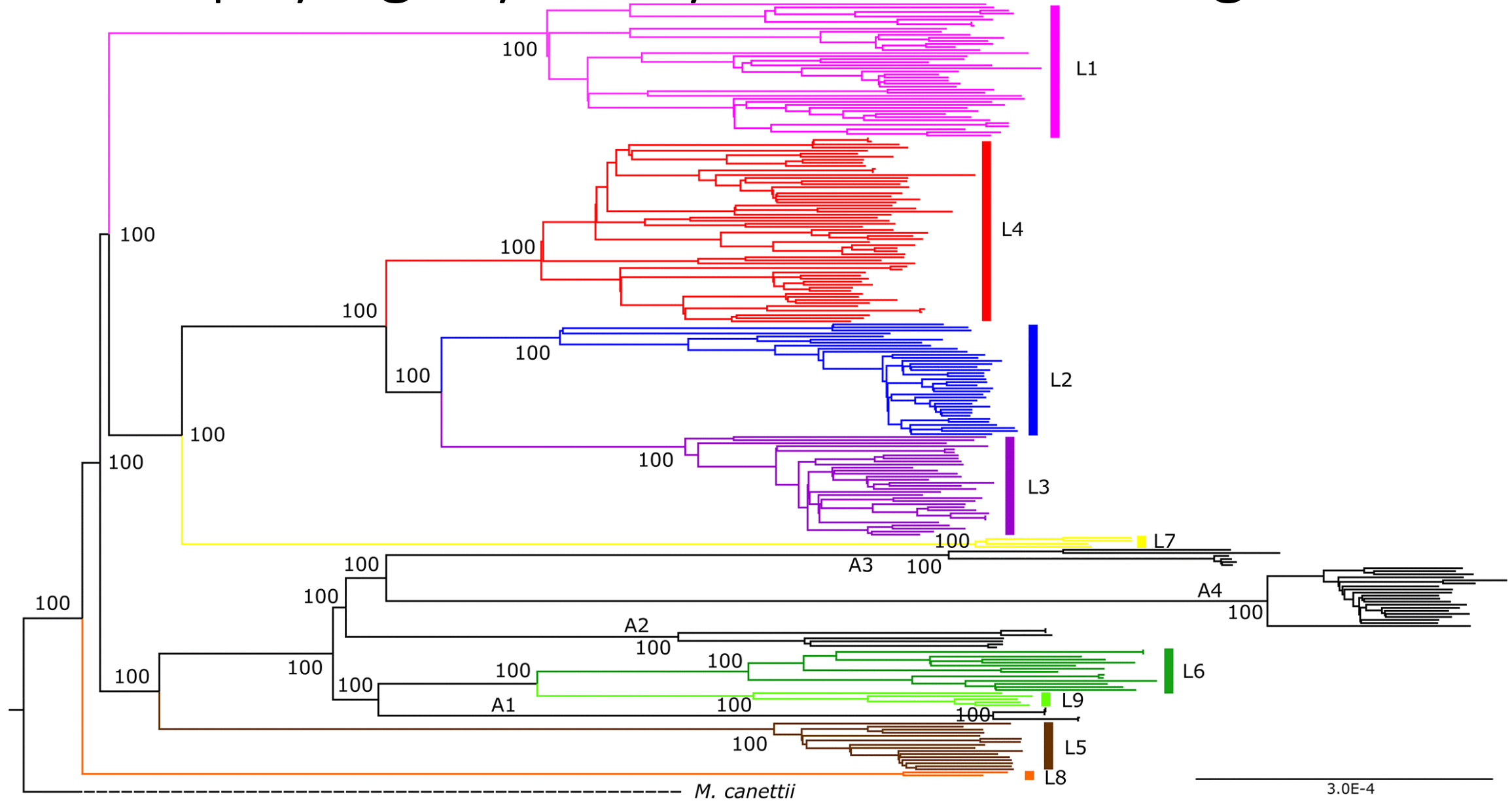
TYK2: strong negative selection in Europe ~3,000 y ago

Kerner, AJHG 2021

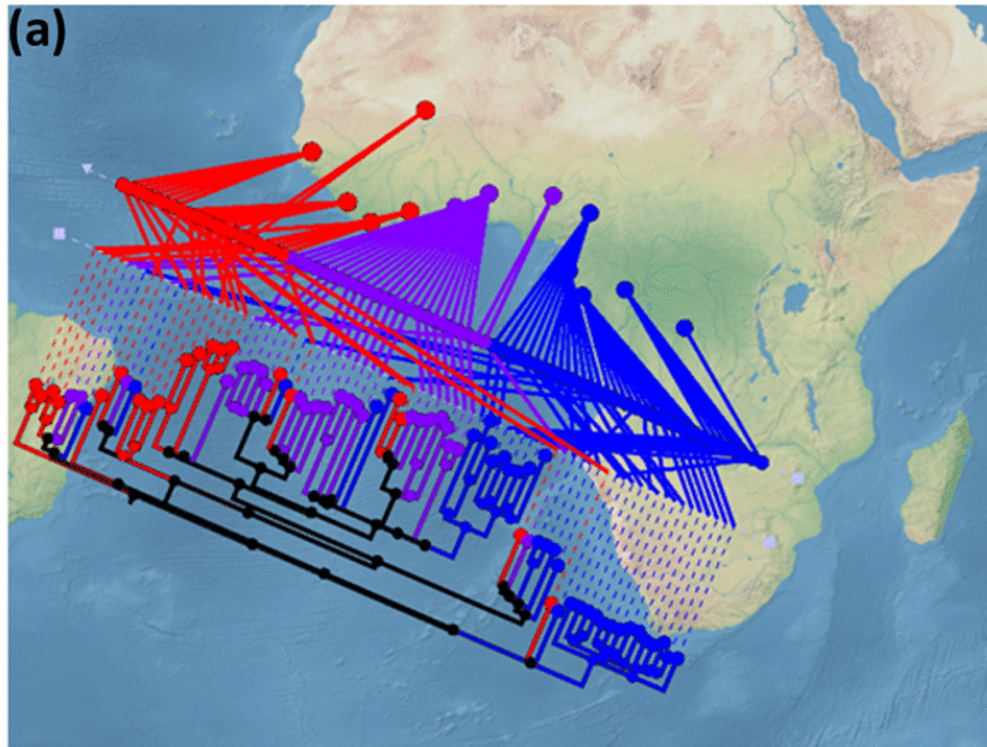


Comas NatGen 2013

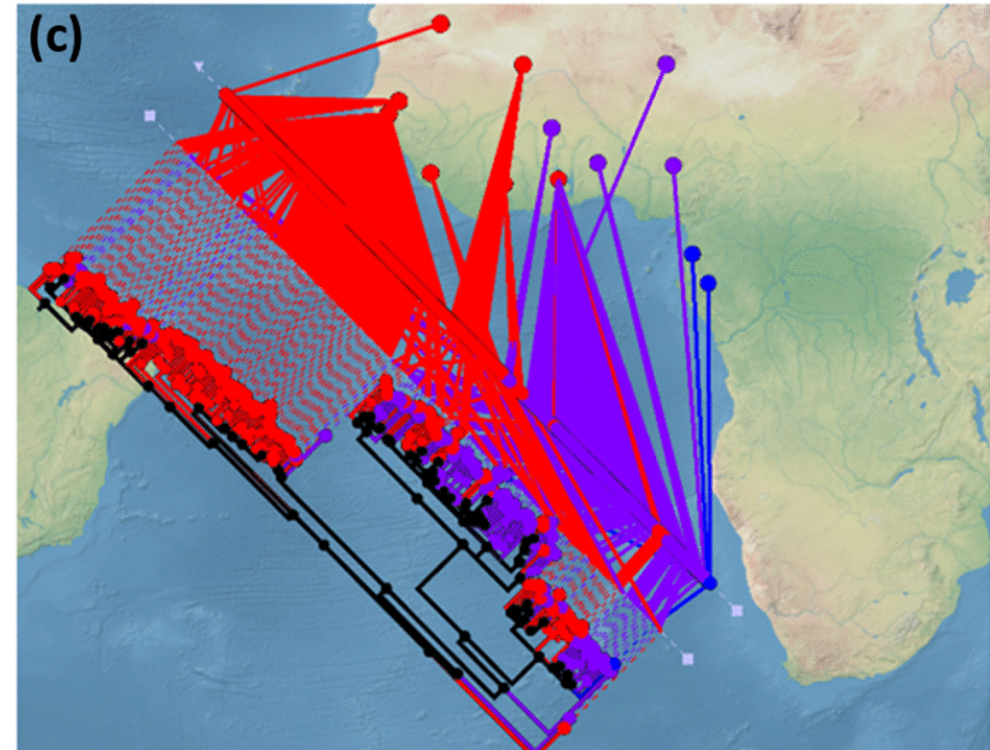
MTBc phylogeny today - 9 human lineages



Marked geographical structure within L6, but not within L5

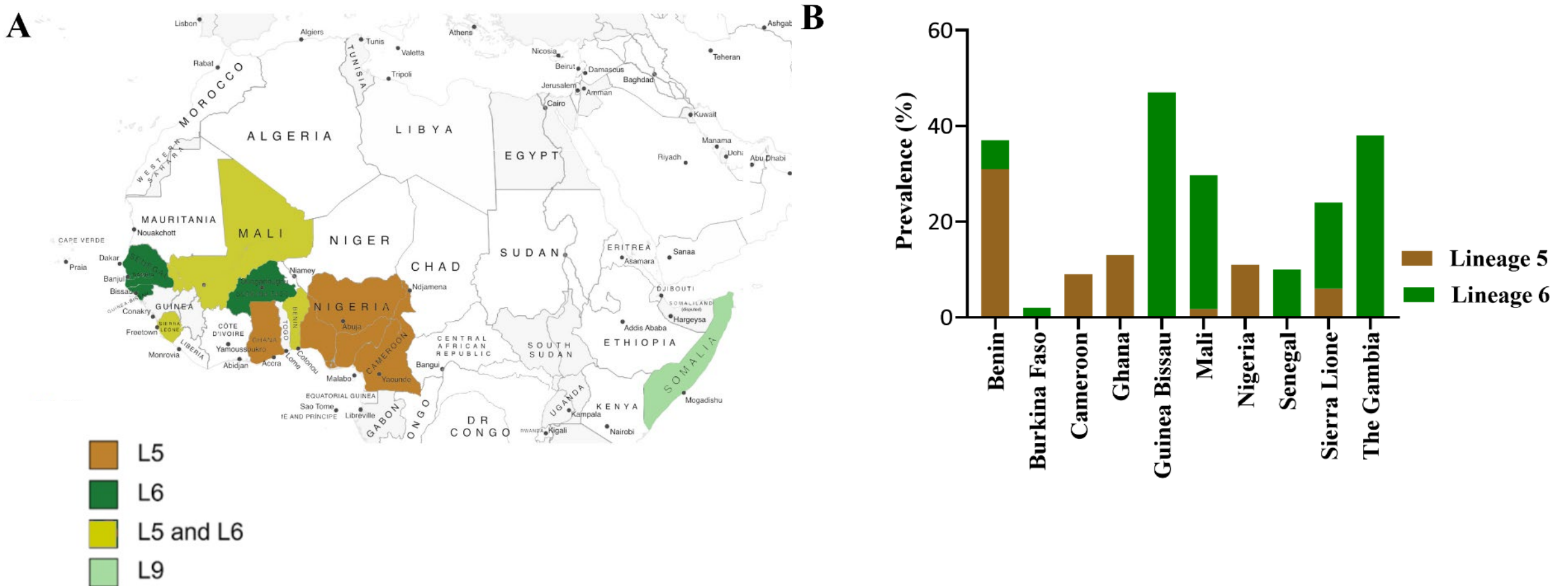


M. africanum West-African 1
MTBc Lineage 5



M. africanum West-African 2
MTBc Lineage 6

M. africanum causes up to 40% of tuberculosis



Tuberculosis caused by *Mycobacterium africanum*: Knowns and unknowns- Silva et al PLoS Path 2022

Dysgonic growth limits pDST



L4 - eugonic

L5, L6 - dysgonic

negative

Photo by N'Dira Sanoussi

L6 grows microaerophilically in Lebek



Neg

L4-H37Rv

L4

L6

L6

Ofori-Anyinam Tuberculosis 2017

Diagnostic differences

M. africanum (L5 + L6)

vs.

M. tuberculosis L4

Culture slower, ↓ yield

L6 microaerophilic (~ *M. bovis*)

**MTP64
based**

24% false negative L5
nSNP in MPT64

identification

21.6% false negative L6

Underexpression of MPT64

→ misclassification as Non-Tuberculous Mycobacteria

L5 and L6 are less likely to grow in culture

Smear + TB patients in Benin

Direct
genotyping

paired analysis

Indirect
genotyping

Culture- LJ

53 neg

146 pos

Spoligotype analysis

Success: **98.5%** (196/199)

73.4% (146/199)

Primary isolation culture success

L4 **89.3%** (566/623)

L5 **78.2%** (230/294)

L6 **61.0%** (50/82)

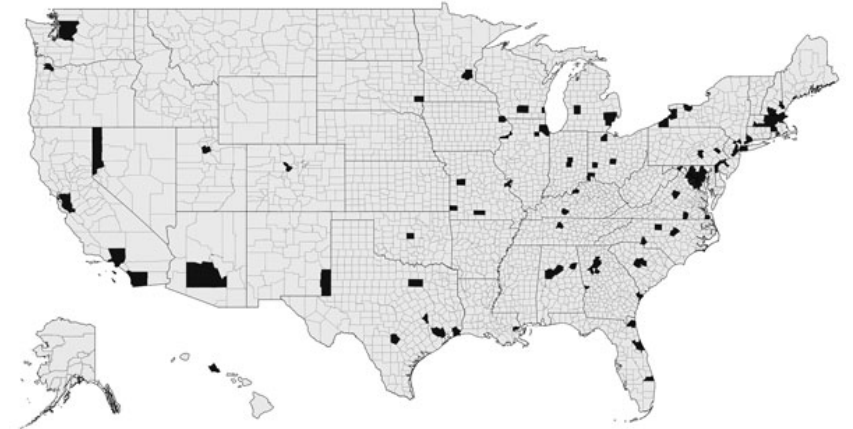
Differences in clinical presentation

M. africanum (L5 + L6)

vs.

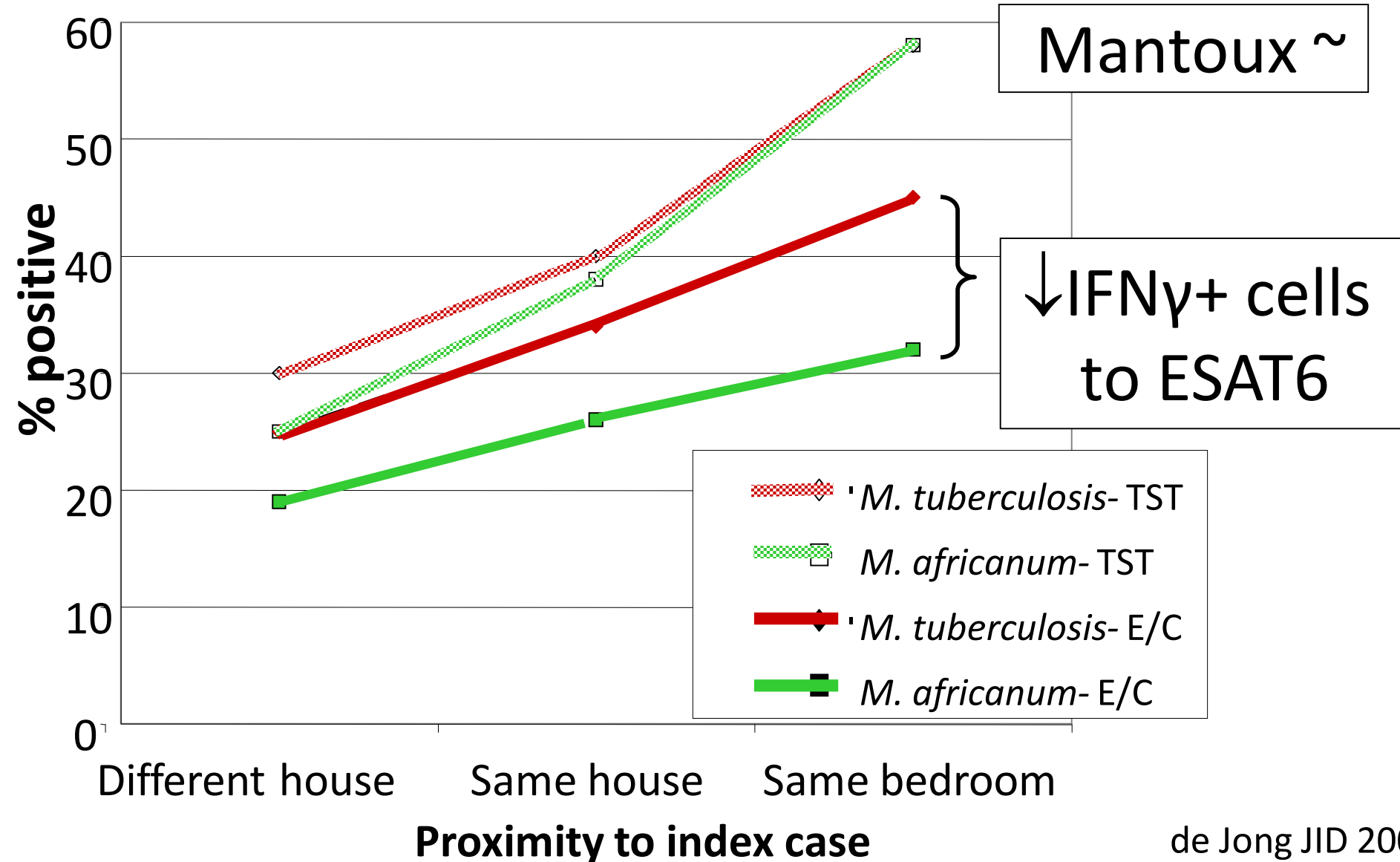
M. tuberculosis L4

- ↑ Age
- Delayed presentation
 - Prolonged duration of cough
 - ↓ BMI
 - ~ More extensive radiographic disease
- Immunocompromise
 - HIV
 - Diabetes

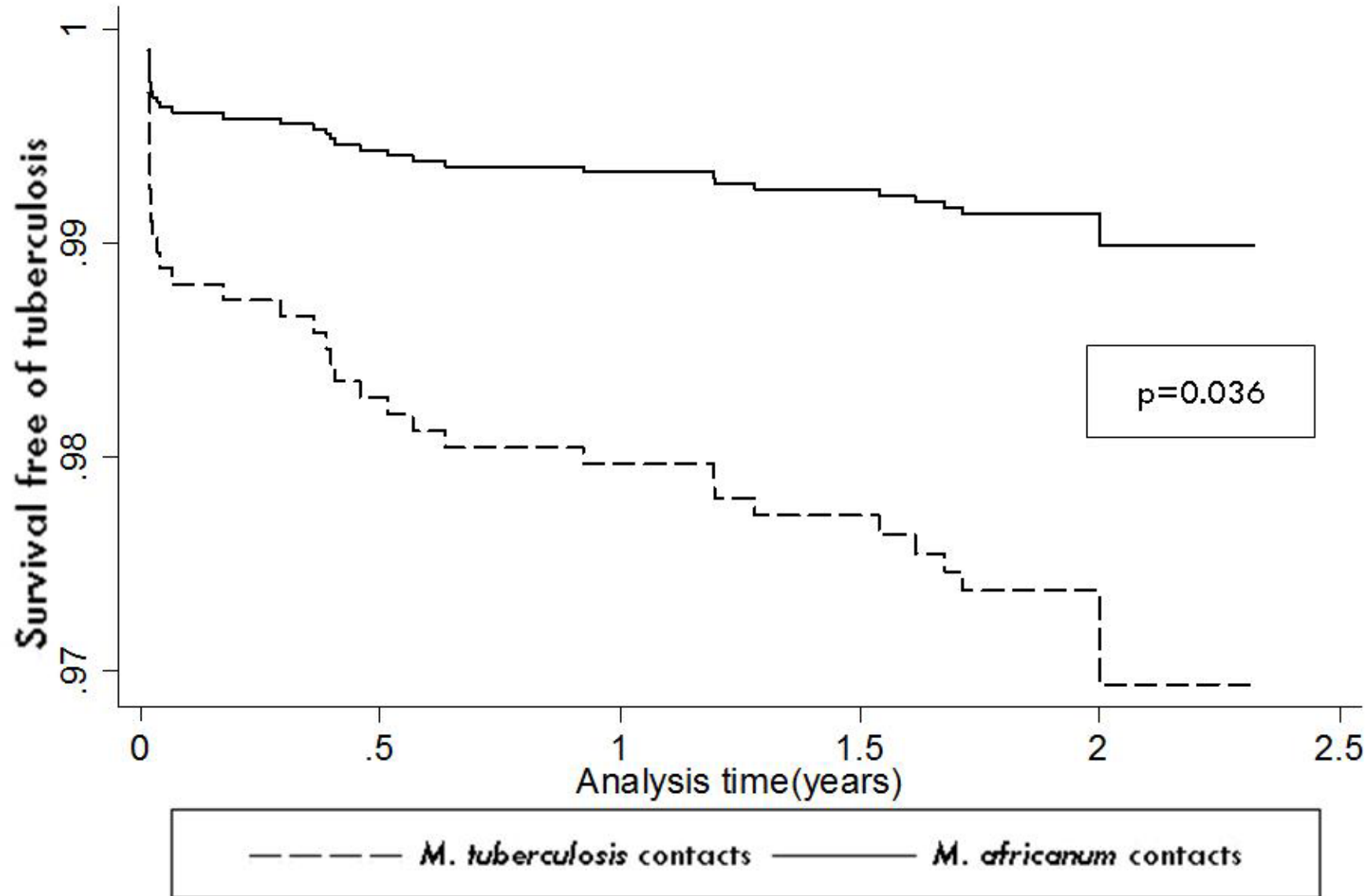


- 0.4% of all TB, West African immigrants
 - ↑ HIV
 - ↑ extrapulmonary disease
 - ↓↓ secondary case rate

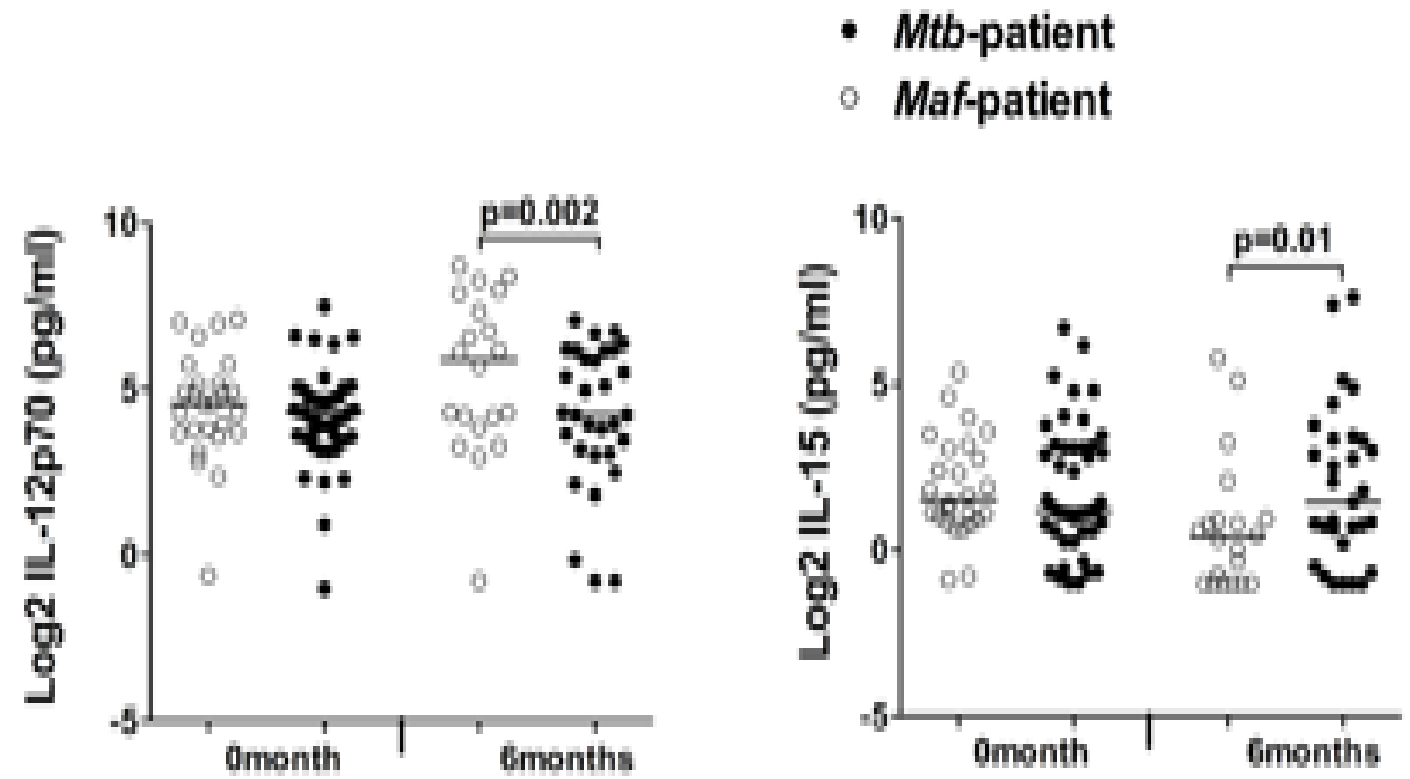
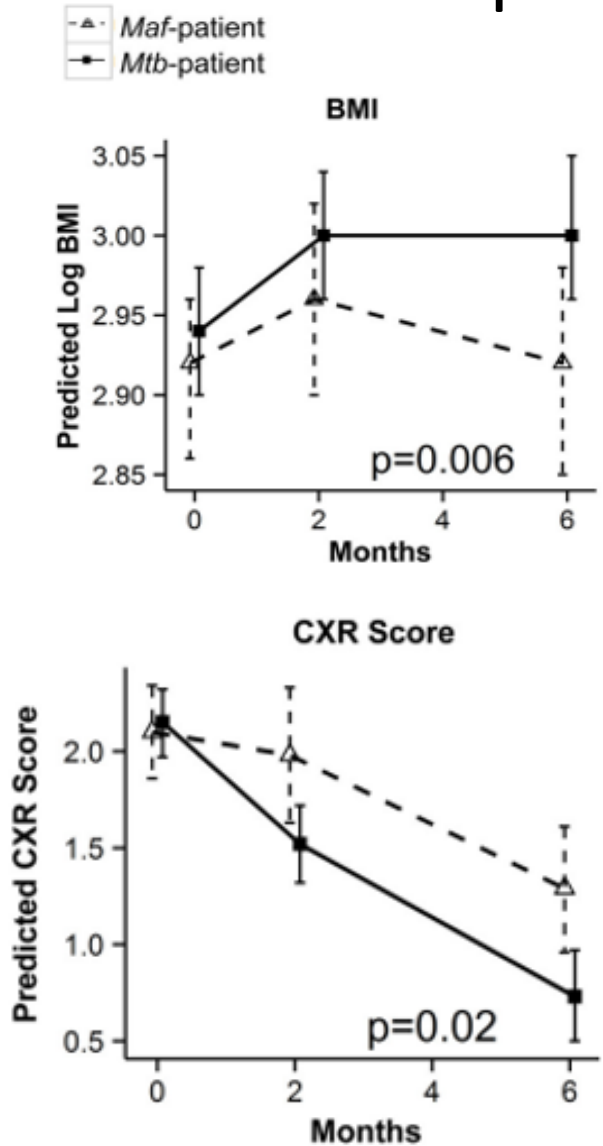
Transmission *M. africanum* L6 = *M. tuberculosis*



Progression to disease *M. africanum* L6 < *M. tuberculosis*



M. africanum L6 infected patients show slower response on treatment ~ Mtb



L6: smears convert slower on treatment in Bamako

Fig 2A.

Auramine microscopy

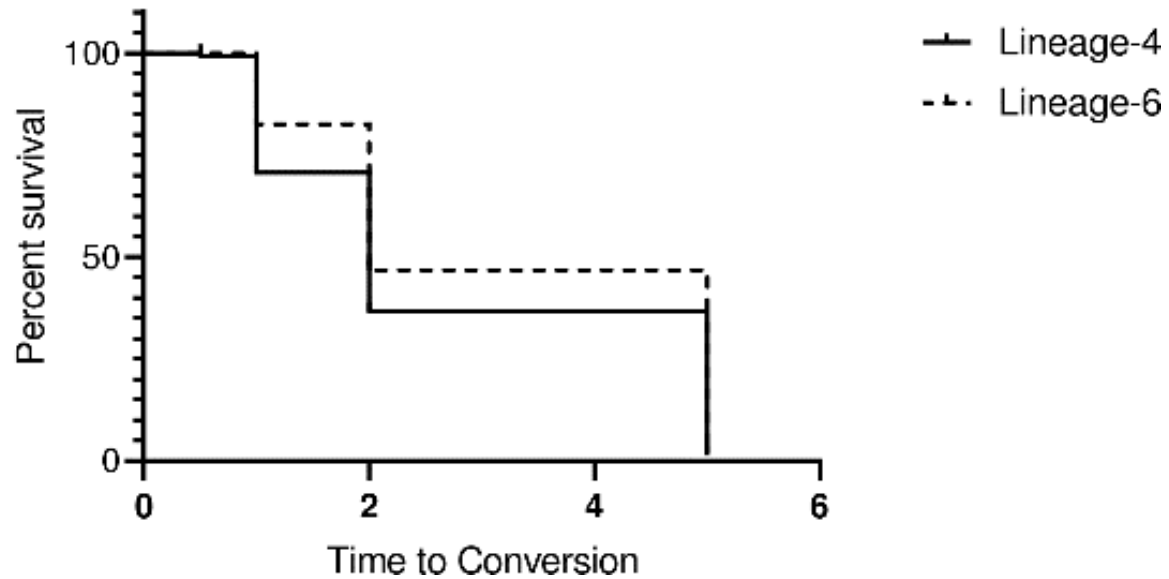
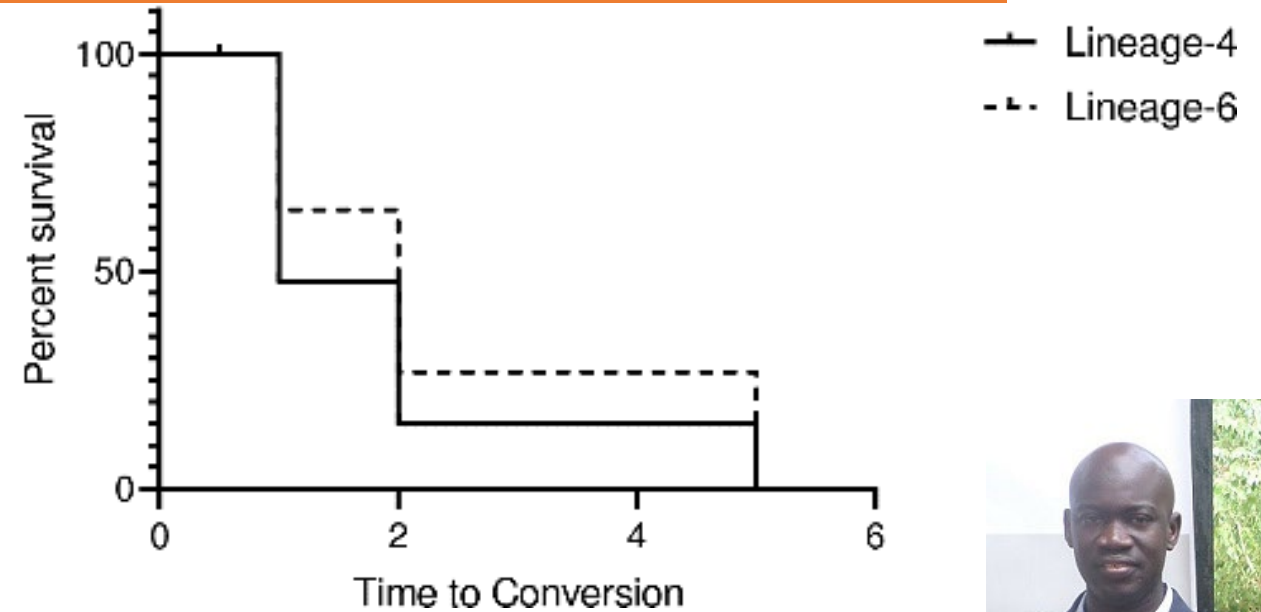


Fig 2B.

Fluorescein Diacetate "vital stain" microscopy

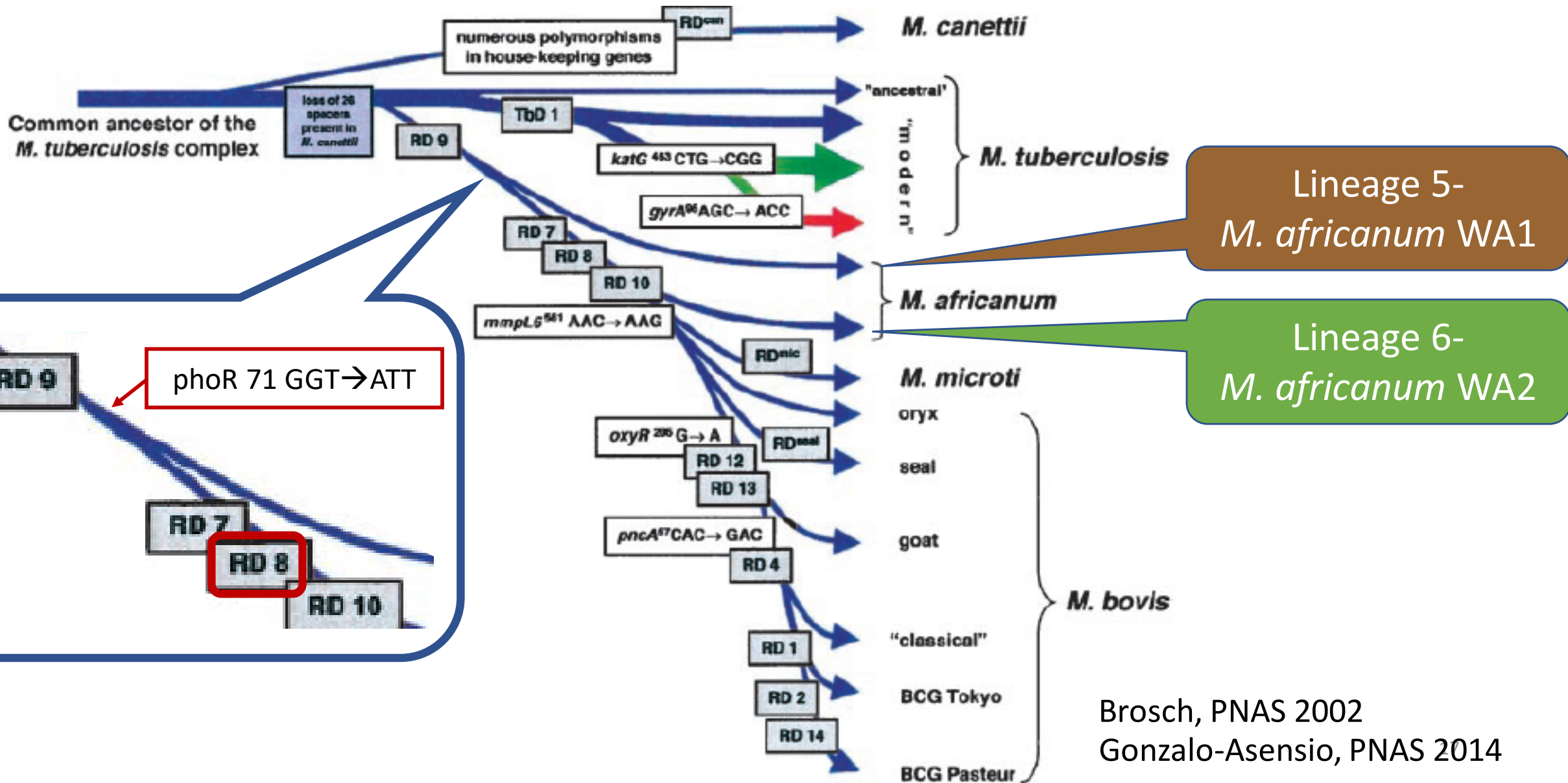


Yet, L6 is underrepresented in retreatment patients

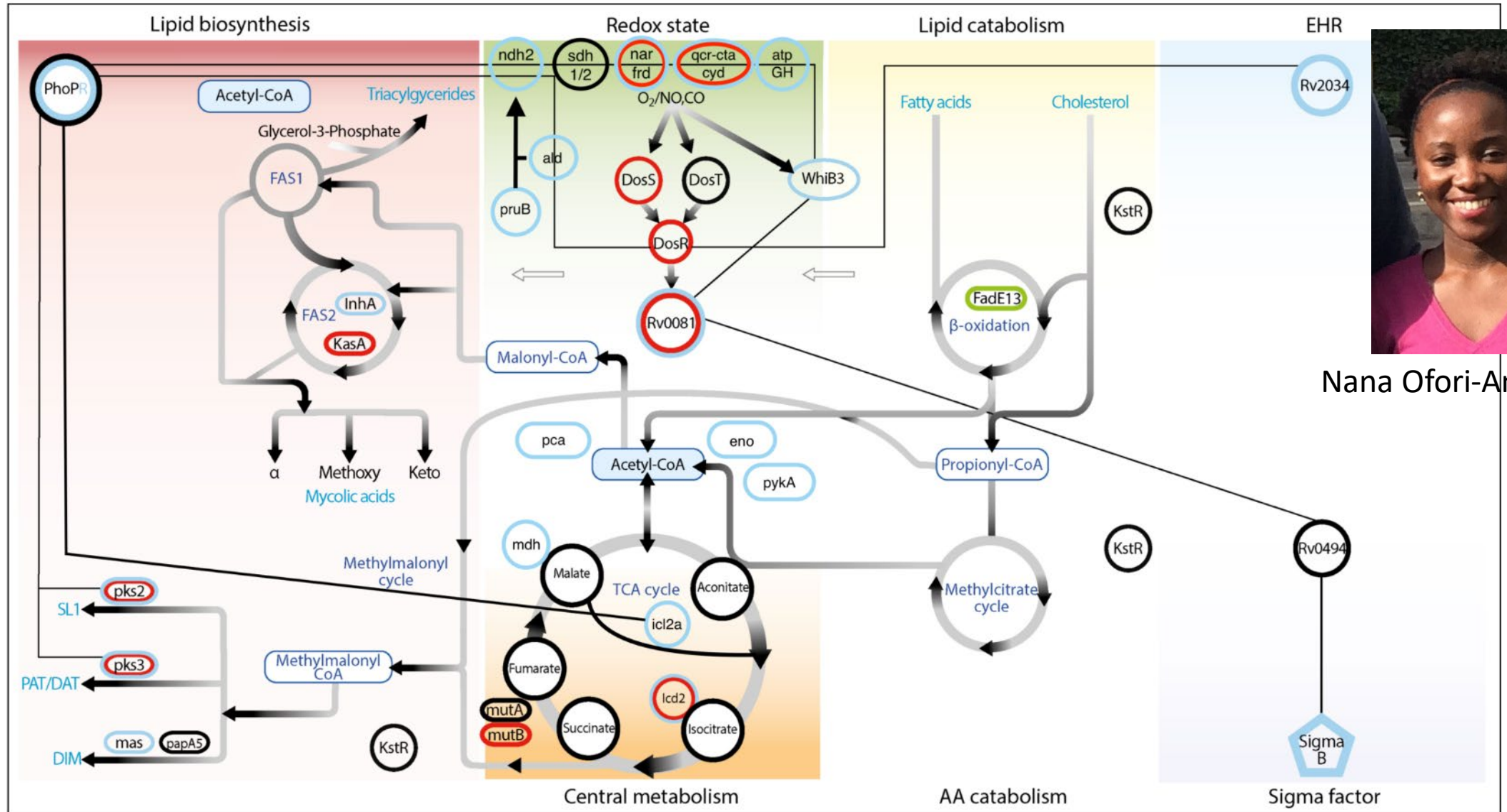


Bassirou Diarra

PhoR mutation shared by L5 + L6 + *M. bovis*-ESX1 function rescued by RD8



L6: lipid catabolism, less respiration



Nana Ofori-Anyinam

Modified after Galagan et al, Nature, 2013

Figure by Nana Ofori-Anyinam, based on Gonzalo-Asensio PLoS1 2008; Ofori-Anyinam Tuberculosis 2017; Galagan Nature 2013

● significantly downregulated
● significantly upregulated
○ n5n mutated
 no significant difference

Why has *M. africanum* not disappeared yet?

Burkina Faso

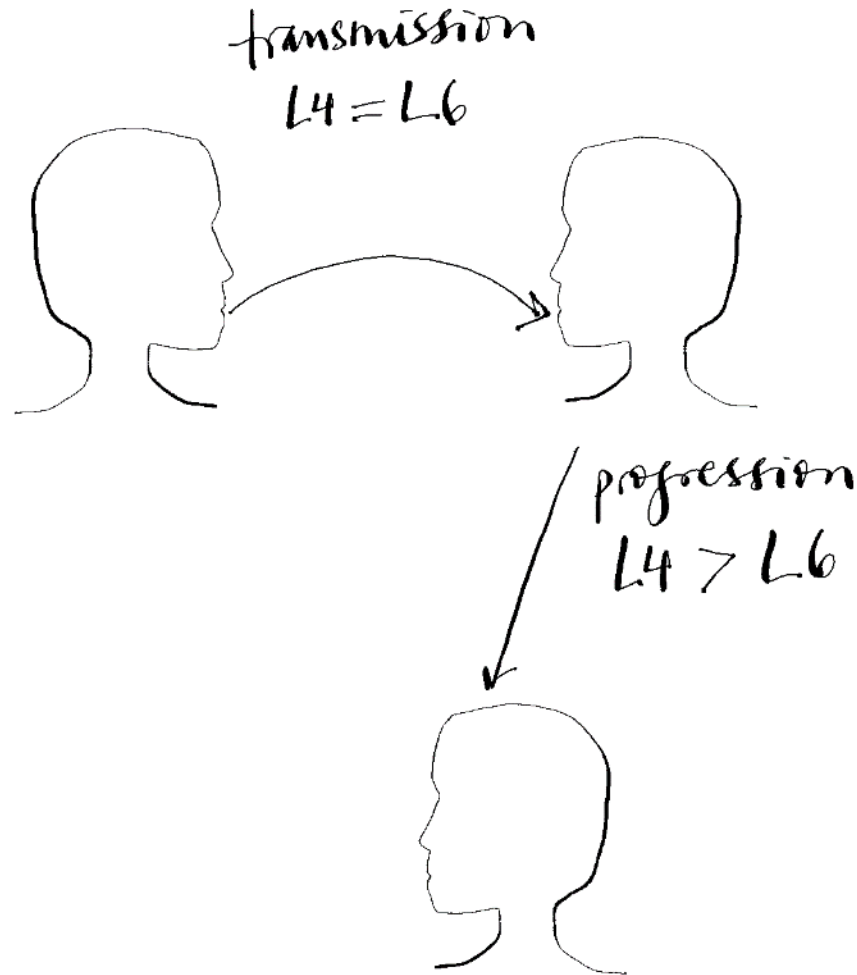
Stable ~20%
1989-2012

Ghana

↓ 2008: 29.2%
↓ 2016: 18.3%

Cameroon

↓ 1971: 56.0%
↓ 2014: 2.3%

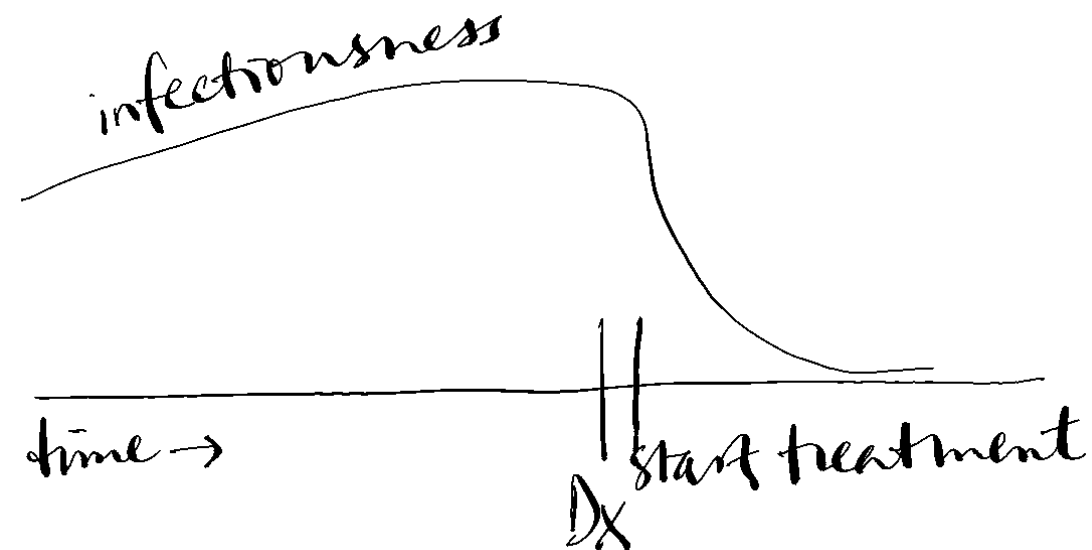


→ Longer infectiousness?

- Late presentation

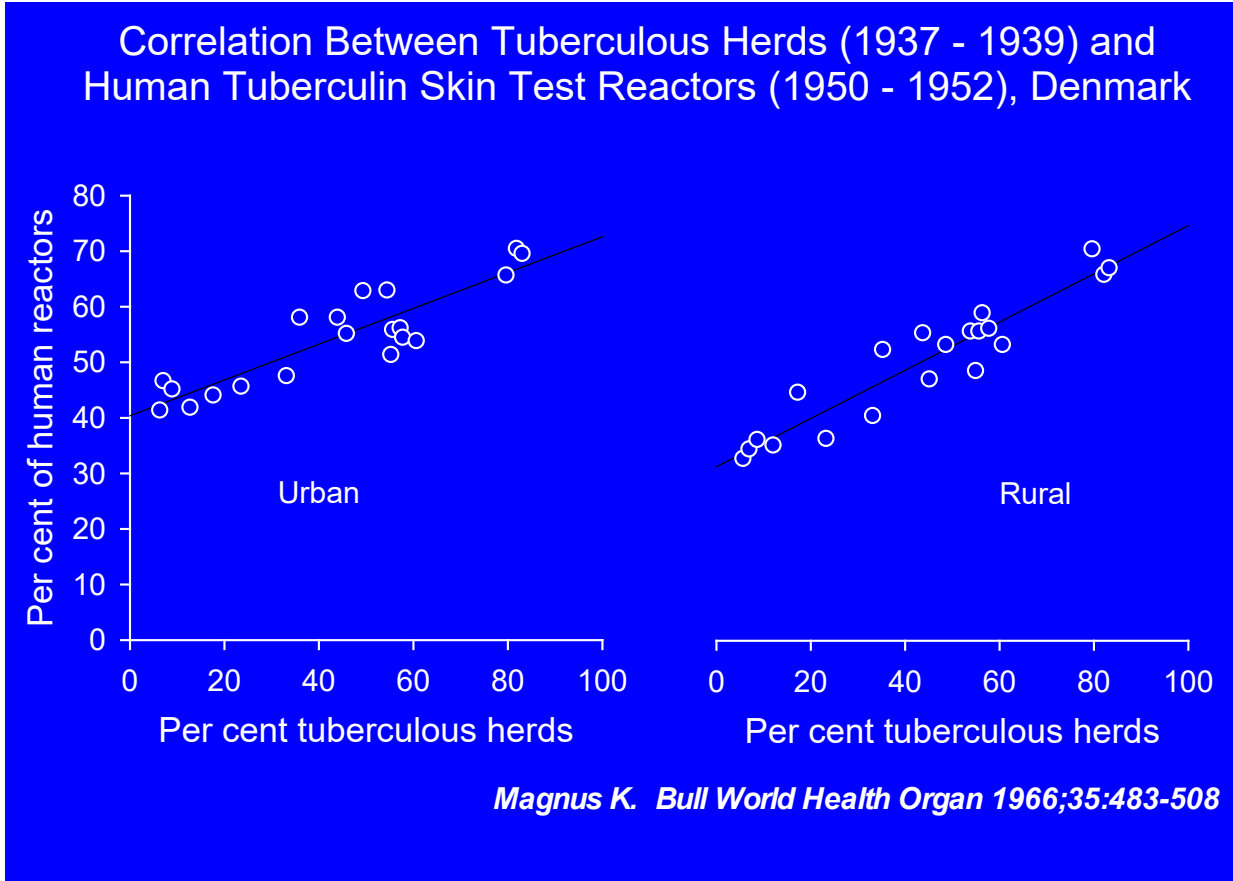
→ Host adaptation

→ Animal reservoir?



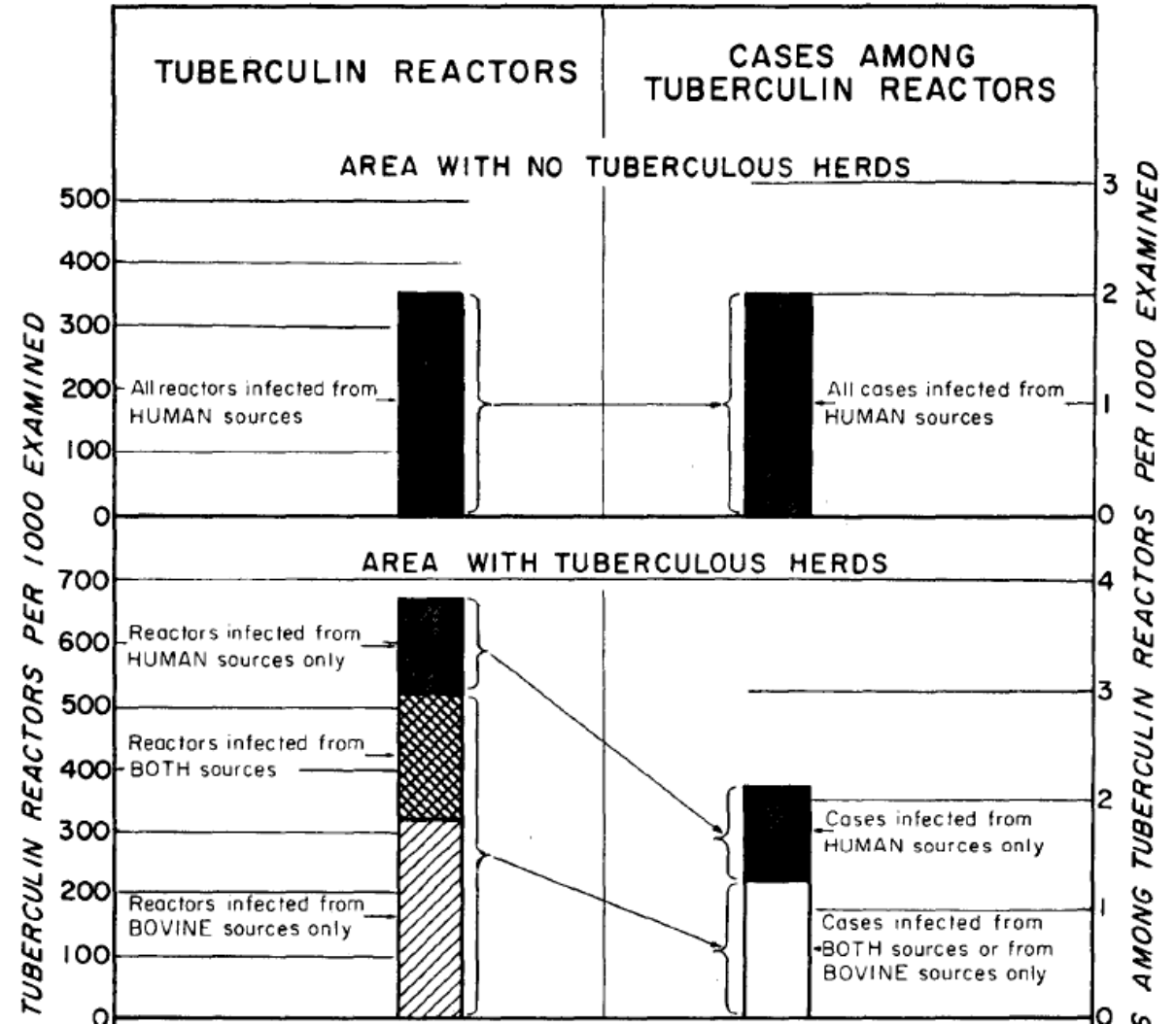
M. bovis - lower progression to human TB disease

relatively large reservoir of infected rural population in Denmark ~ 1950



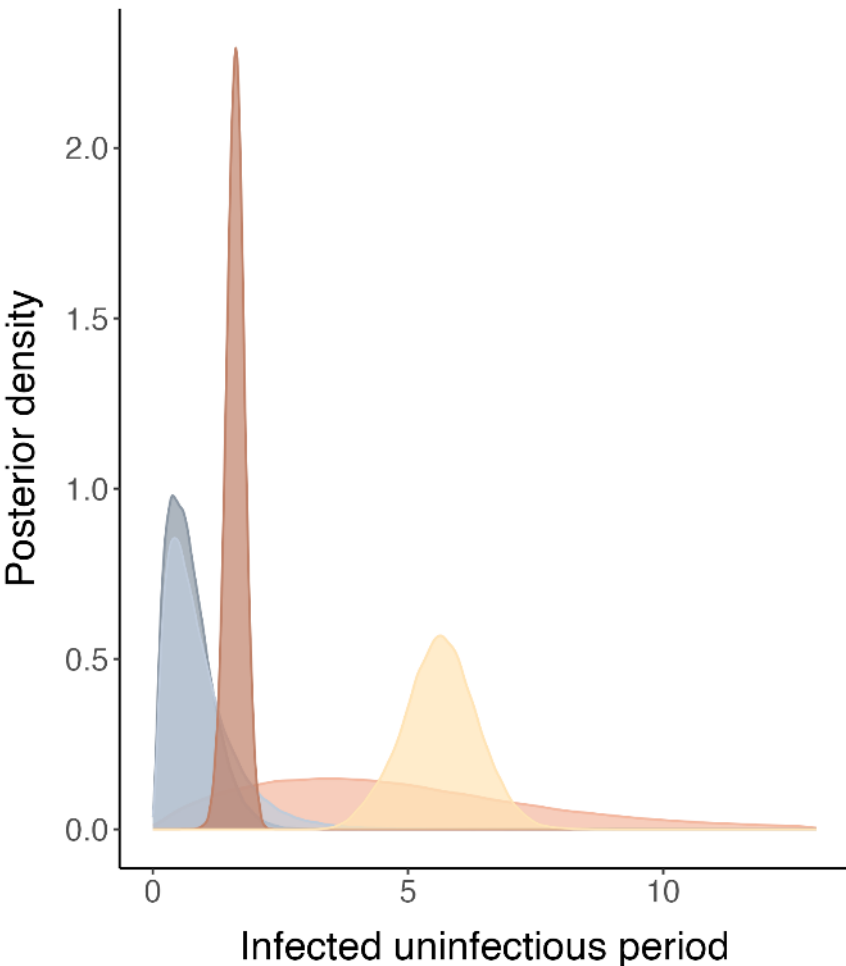
Slide by Hans Rieder

FIG. 9
SCHEMATIC FIGURE ILLUSTRATING BASIS FOR ESTIMATION OF MORBIDITY RATES AMONG REACTORS INFECTED FROM HUMAN AND BOVINE SOURCES

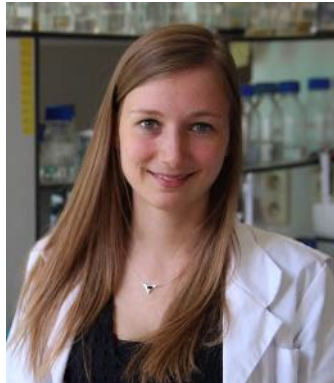
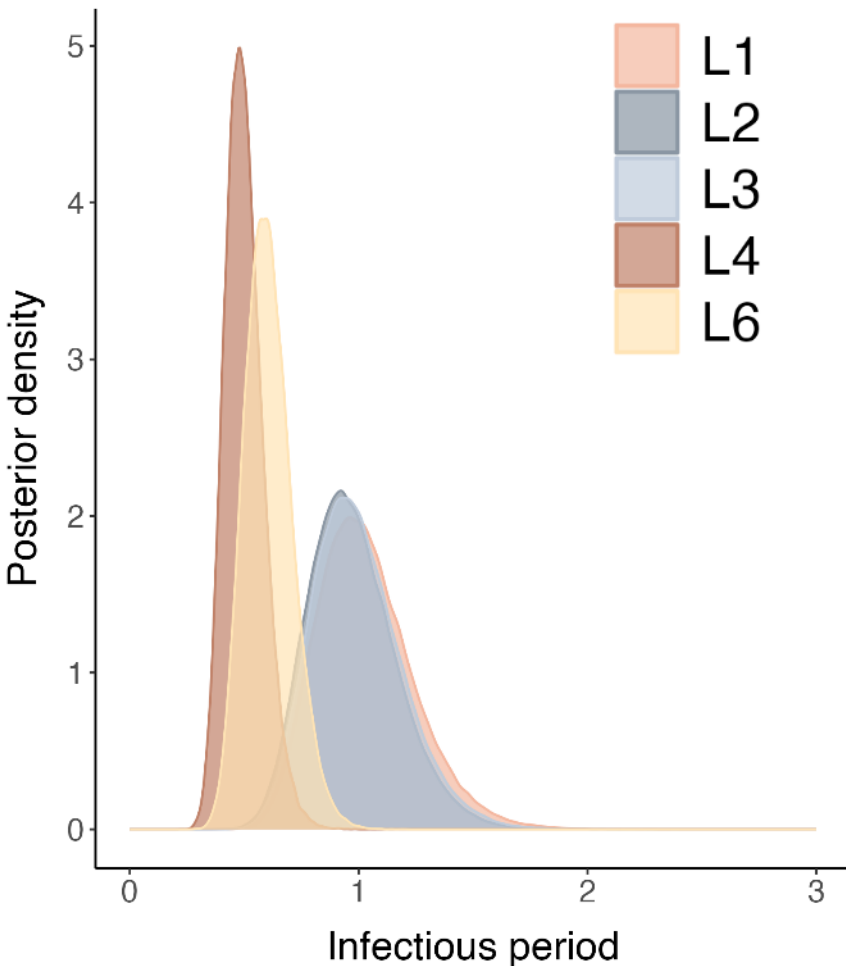


Preliminary phylodynamics- The Gambia: latency longer for L6 than L4

Infected uninfected period



Infectious period



Etthel Windels

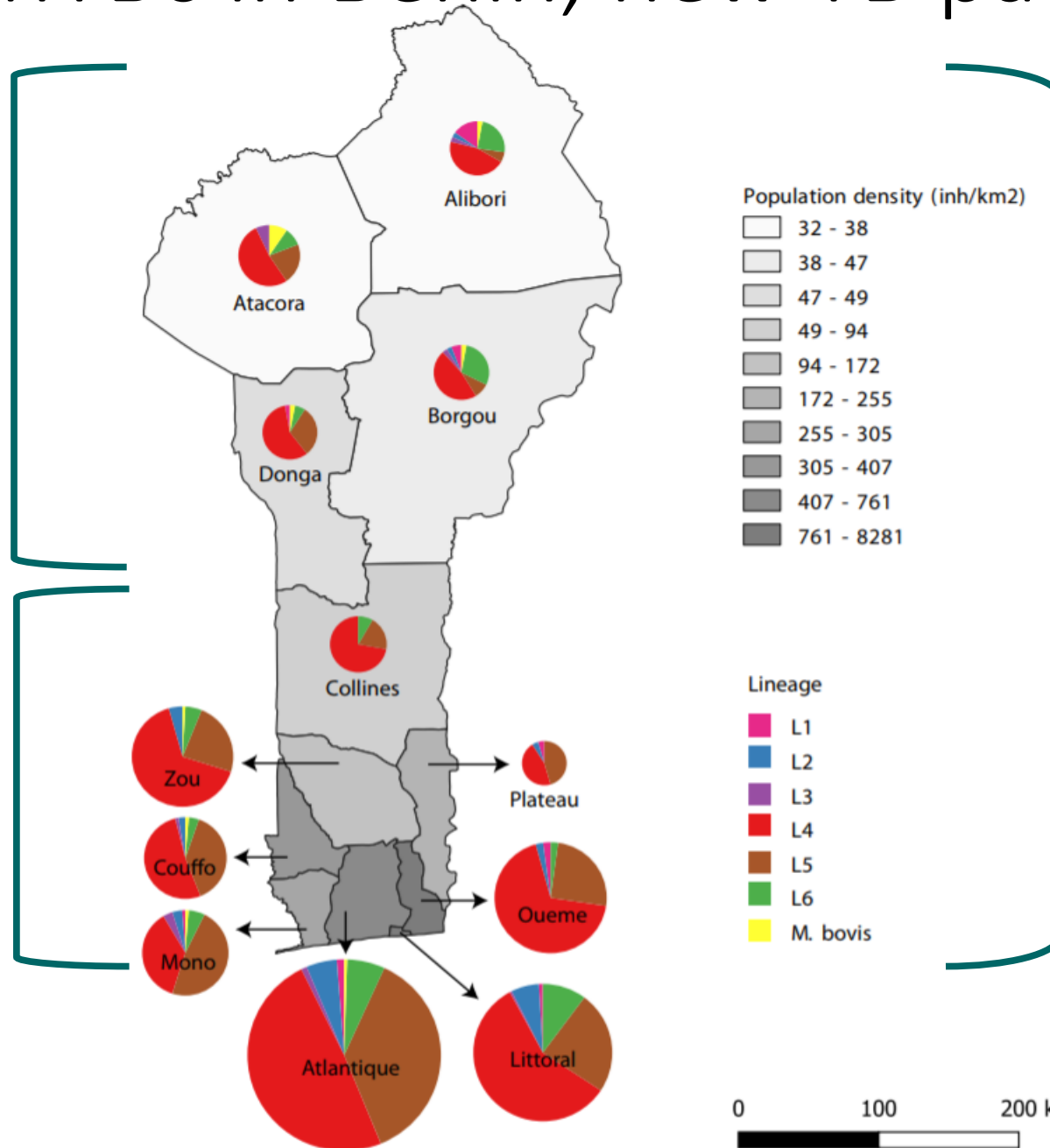


Boatema Ofori Florian Gehre



Conor Meehan Martin Antonio

MTBc in Benin, new TB patients



N'Dira Sanoussi,
unpublished

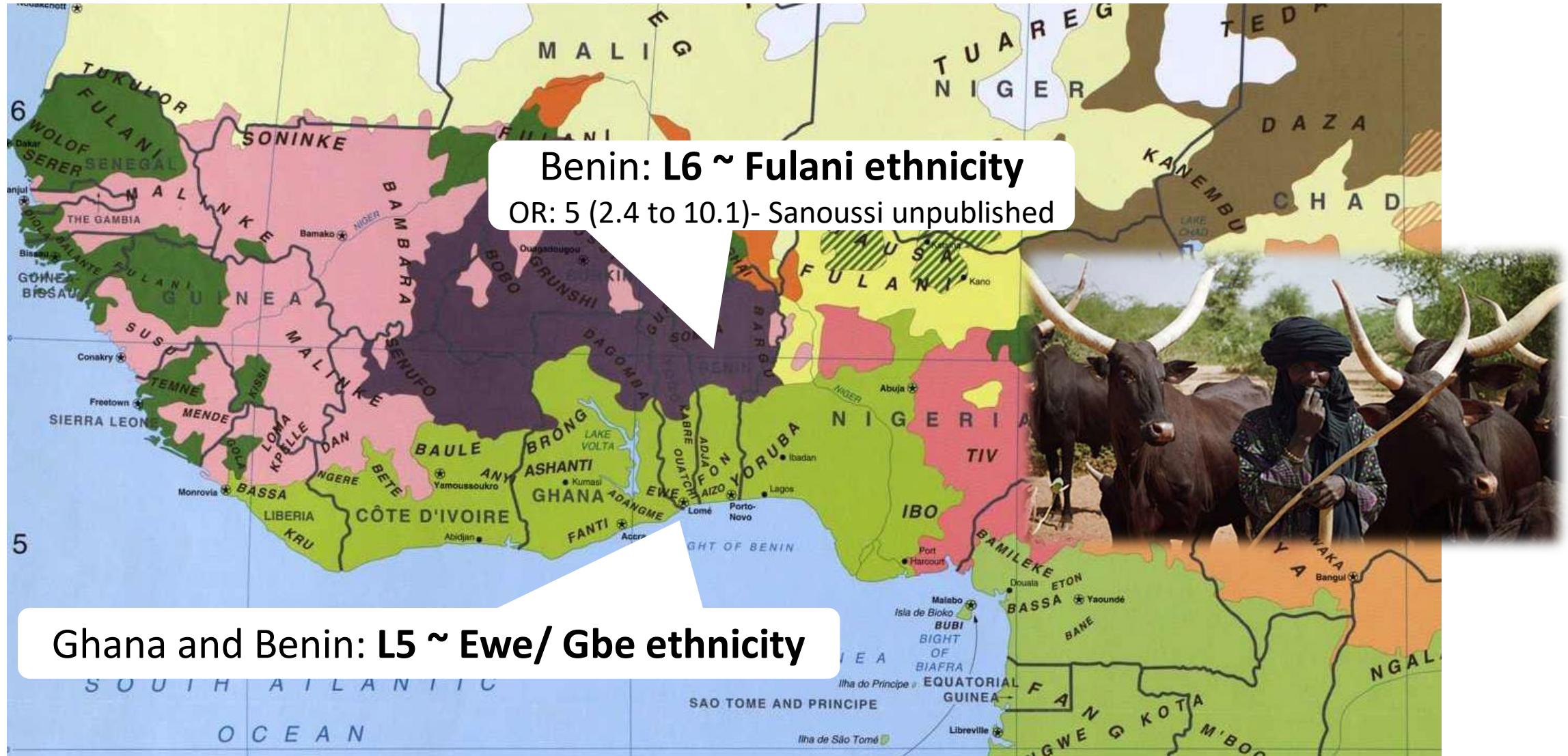
L6 - 8.1%
> north East

L4- 52.9%

L5 - 31.1%
> on coast

L2- 3.8%
> urban

Host-pathogen adaptation



5-lipoxygenase (*ALOX5*) variant ~ L6

Autophagy related human immunity-related GTPase M (*IRGM*) variant ~ L5/L6

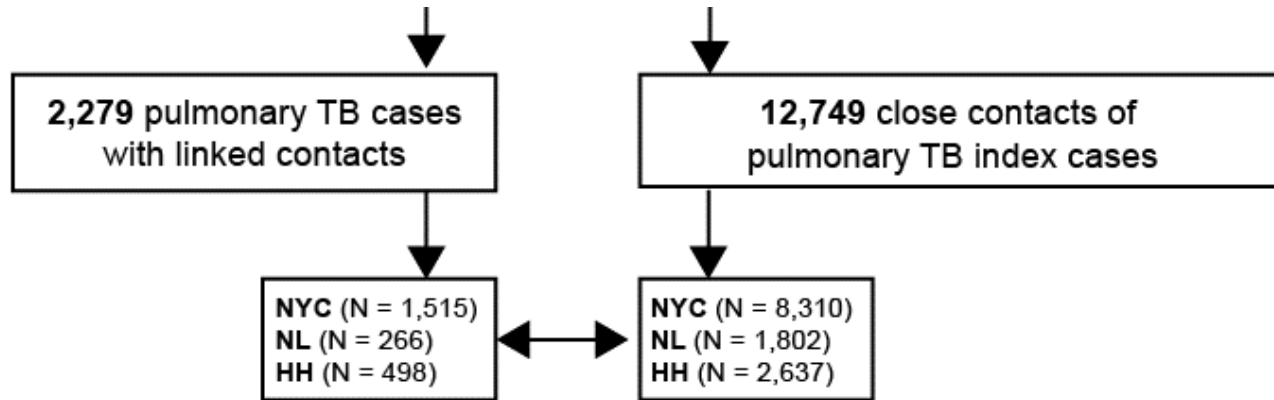
Mannose Binding Lectin (*MBL*) ~ L4

reviewed in Tientcheu 2017 EJI

Sympatric versus allopatric transmission

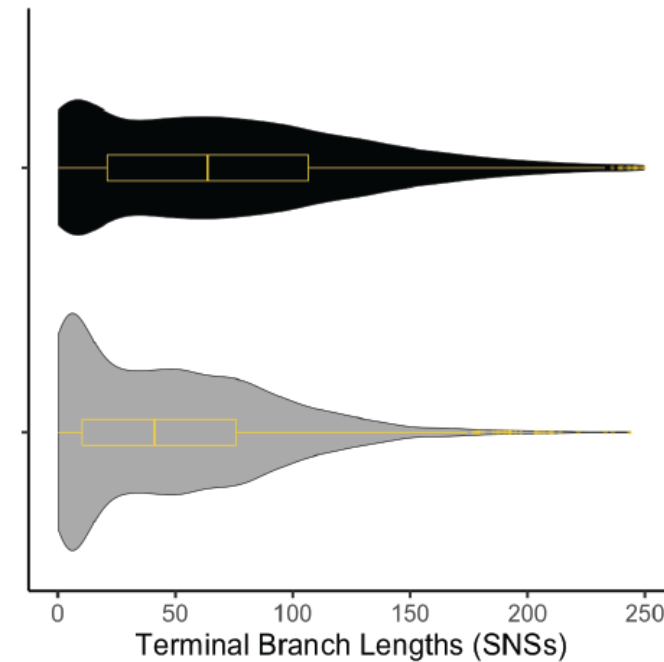


Matthias Gröschel
2022, medRxiv



Geographically restricted
(Mtb L1, L2 ancestral, L3,
L4 sub-lineages, L5, L6)

Geographically widespread
(Mtb L2 modern, L4)



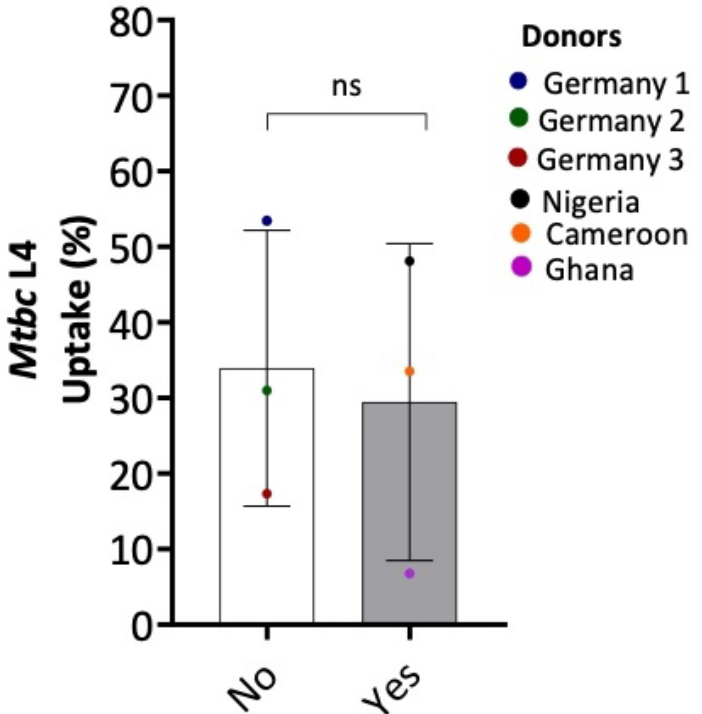
Allopatric host-pathogen exposures had a 32% decrease in the odds of infection among contacts compared with sympatric exposures

Geographically restricted lineages yield significantly fewer secondary active TB cases among close contacts controlling for index characteristics

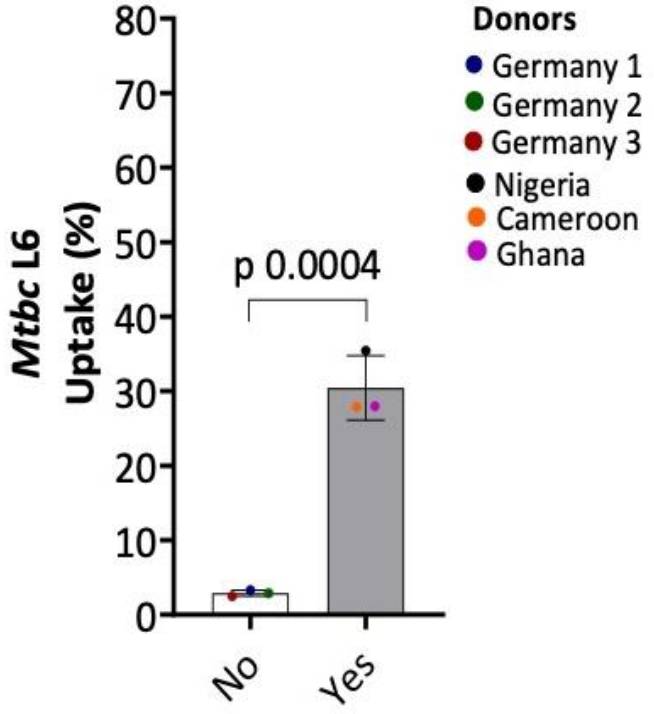
Sympatric strains: > MΦ uptake & growth

Uptake

Lineage 4

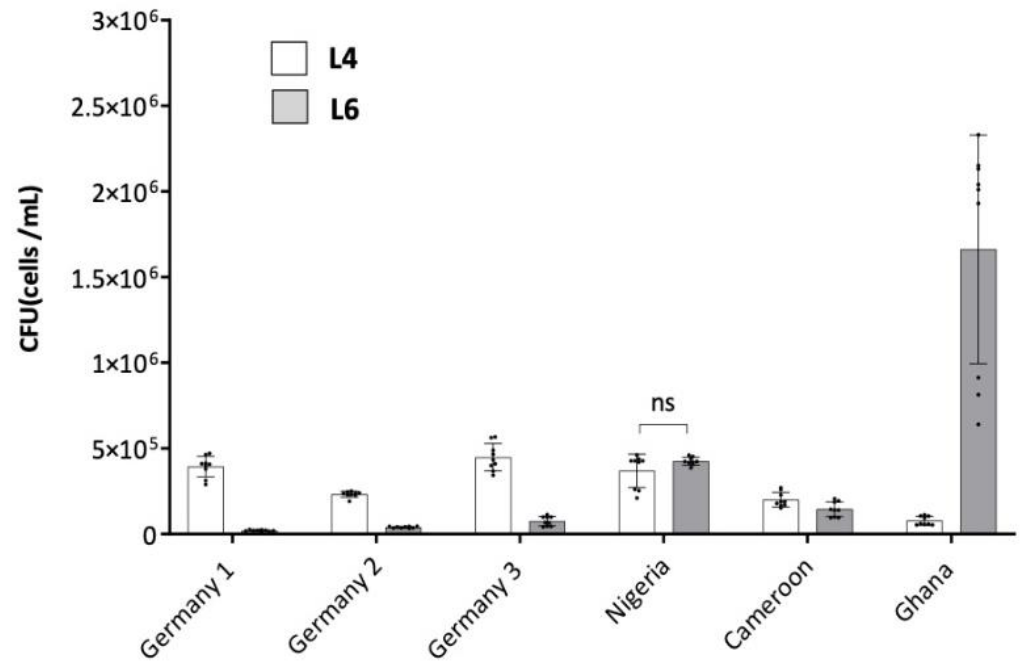


Lineage 6



Ancestry co-localizing with L6

Growth



Human vs animal MTBc members

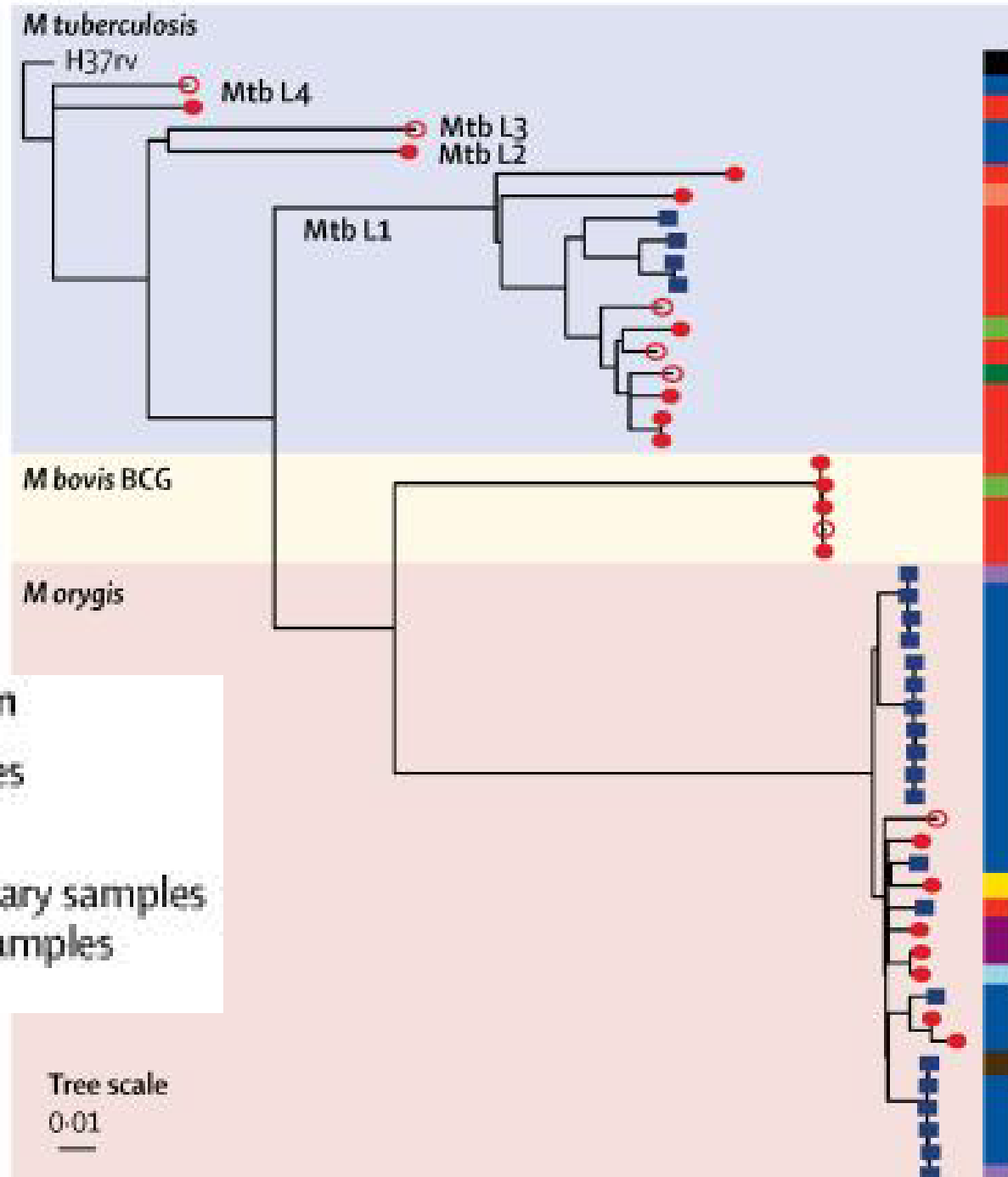
L1 and *M. orygis* in India

Location

- | | |
|------------------|-------------|
| ■ West Bengal | ■ Jharkhand |
| ■ Tamil Nadu | ■ Bihar |
| ■ Andhra Pradesh | ■ Gujarat |
| ■ Madhya Pradesh | ■ Kerala |
| ■ Bangladesh | ■ Karnataka |

Node definition

- | |
|--------------------------|
| ■ Cattle samples |
| Human samples |
| ● Extrapulmonary samples |
| ○ Pulmonary samples |



Treatment for TB was largely developed in India

About half of the strains of M. tuberculosis isolated from patients in Madras with pulmonary tuberculosis are of low virulence in the guinea-pig and have other special characteristics including the presence of an "attenuation indicator" lipid and a phage-typing

~ 50% *M. tuberculosis* Lineage 1

Mitchison 1979 in WHO document on BCG vaccination

↑ TST induration ~ ↓ index case strain virulence

TABLE 2

Mantoux positivity in contacts related to guinea-pig virulence of strains from index patients

| Induration to 5TU (mm) | No. of contacts | Mean guinea-pig virulence of index strains (RIV) |
|------------------------|-----------------|--|
| 0-7 | 197 | 0.76 |
| 8-14 | 231 | 0.73 |
| 15-24 | 86 | 0.70 |
| 25 or more | 54 | 0.69 |

Subclinical TB a/w ancestral lineages?
→ Compare lineages between strains derived from active case finding (incl prevalence surveys) versus passive case finding - Marcel Behr

1.2 An association was found, that just failed to attain statistical significance, between the guinea-pig virulence of strains from index patients and Mantoux positivity of their contacts. (Table 2; 1) The hypothesis is that attenuated strains might cause more chronic disease and therefore infect contacts more often.

Tree scale: 0.0001

Secondary case rate:

- ↑ younger age
- ↑ higher smear grade
- No difference for 'elusive' / borderline *rpoB* mutants vs common *rpoB* mutants
- ↑ for strains with compensatory mutations in *rpoA/B/C*
- **L1<L3<L2<L4**

Borderline *rpoB* mutation

rpoA/rpoC mutation (any)

rpoC

rpoA

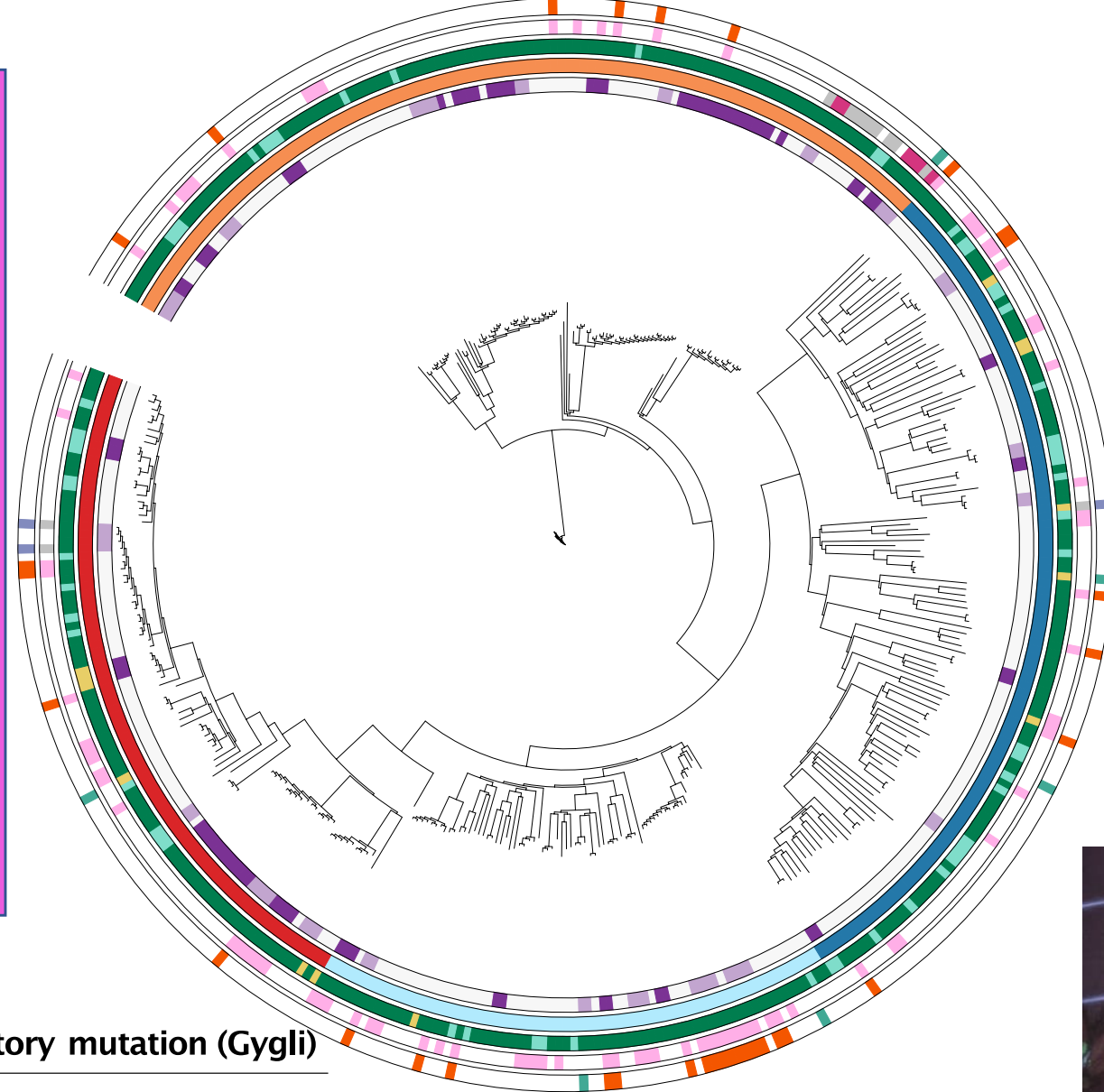
rpoA/C

Compensatory mutation (Gygli)

rpoA

rpoB

rpoC



MDR-TB
Bangladesh
2005-2011

n=394



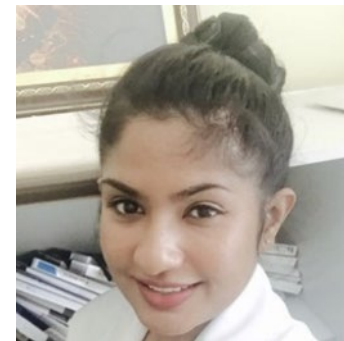
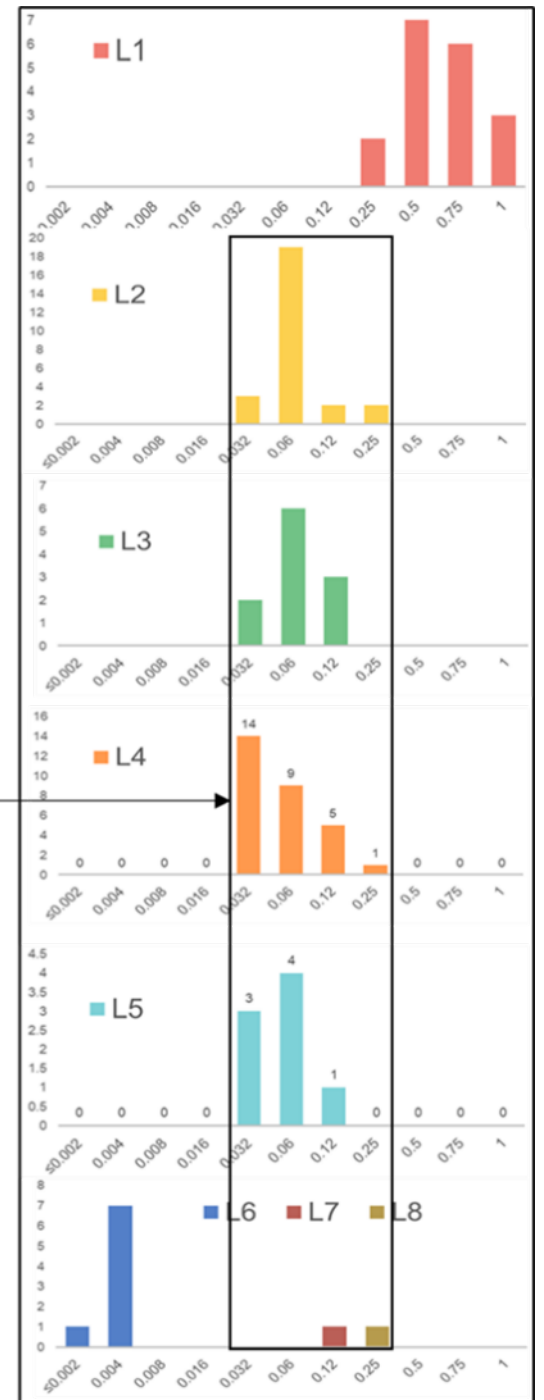
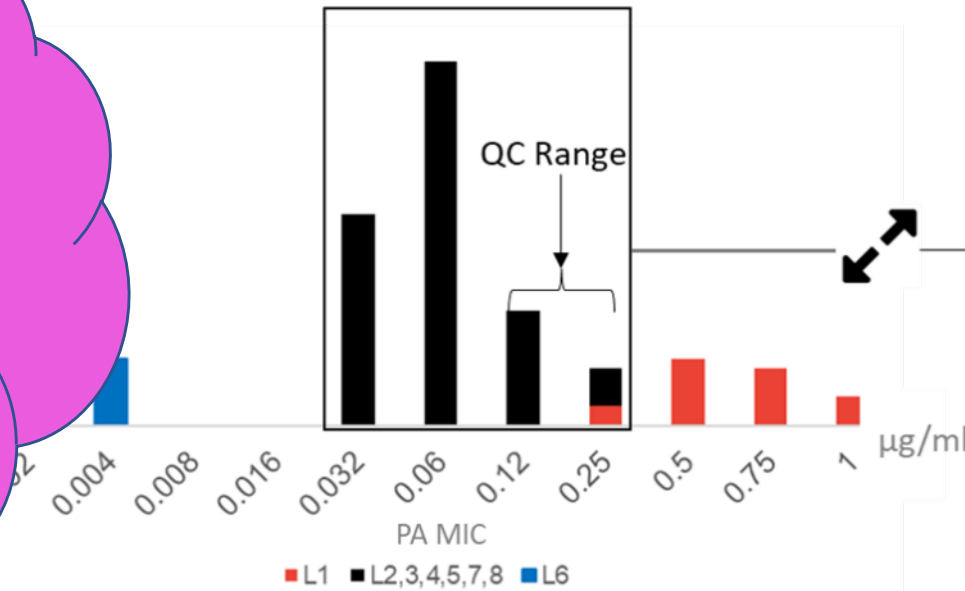
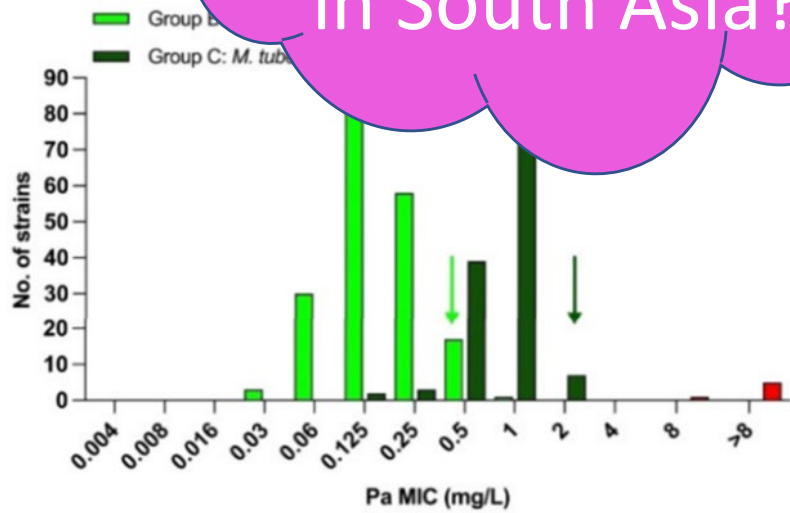
Pauline Lempens, Conor Meehan- submitted

Pretomanid MICs > for L1, < for L6

n=103 (8 lineages, from 46 countries)

Middlebrook 7H11

Genotype lineage before prescribing BPaL regimen in South Asia?

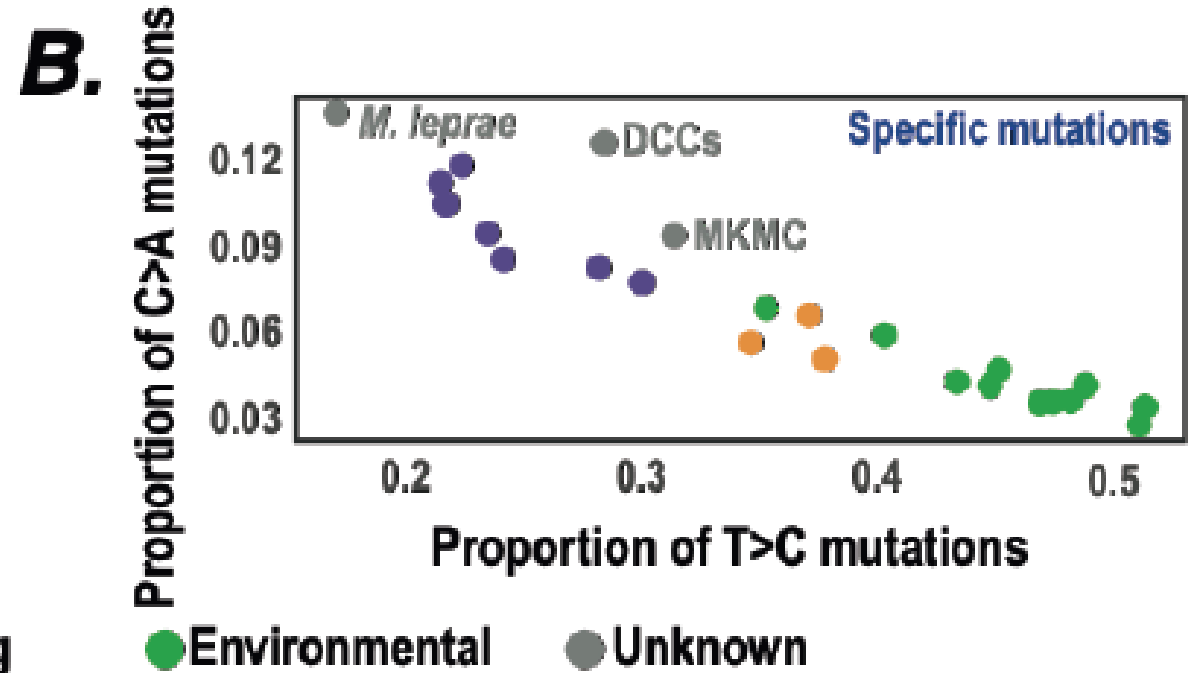
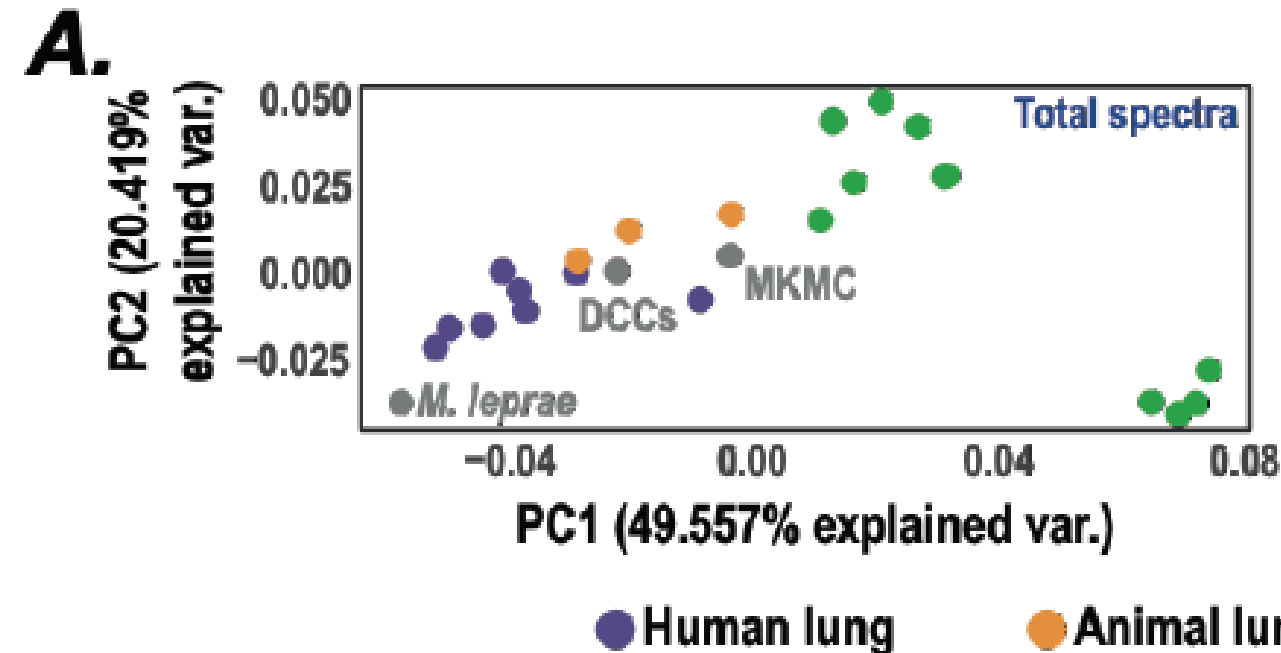
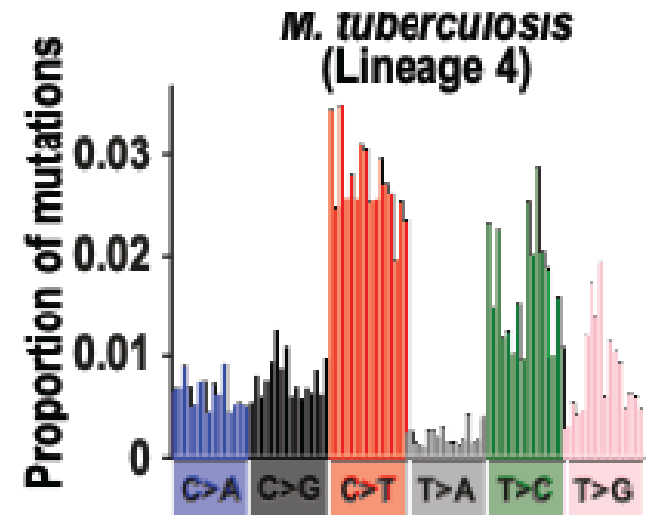


Praha Rupasinge
unpublished data, ITM

Also shown by Bateson et al, in MGIT

Mutational spectra analysis reveals bacterial niche and transmission routes

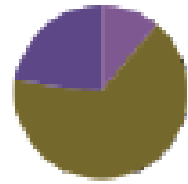
“We find strong evidence that *Mycobacterium leprae* and the dominant circulating clones (DCCs) of *M. abscessus* replicate within the lung”



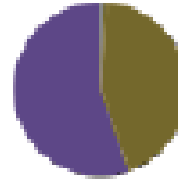
D.

Ancestral-like

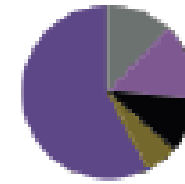
M. canettii



M. abscessus
(environmental)

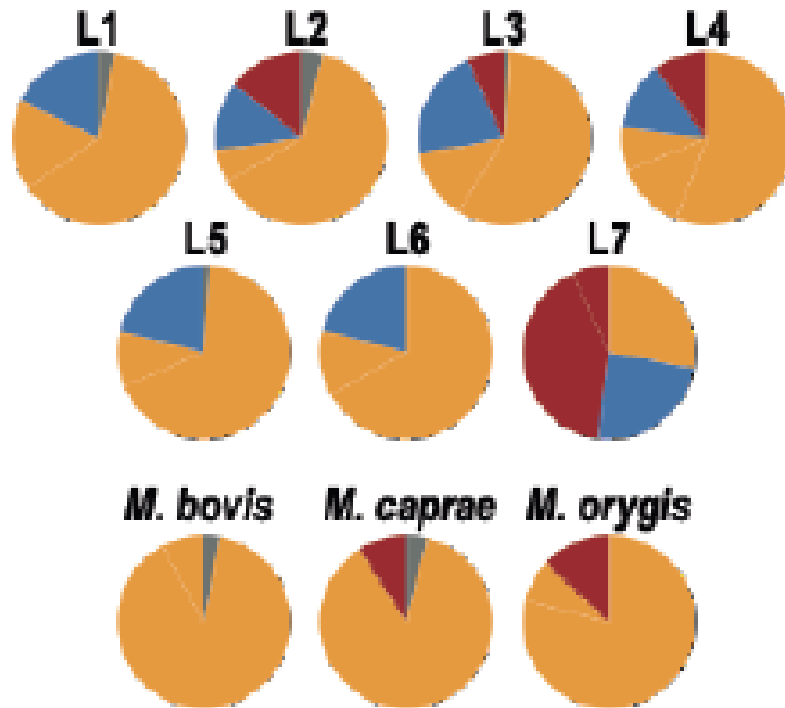


M. kansasii
(environmental)

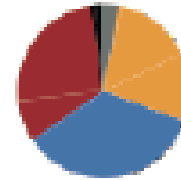


Host-evolved

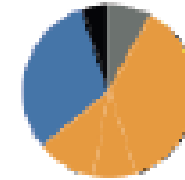
M. tuberculosis



M. abscessus
DCCs



M. kansasii
MKMC



Mutagens

Spontaneous

Nitro-PAH

Alkylating agent

PAH

Smoking

ROS

Other

Unassigned

SNPs in 30k clinical MTB isolates mapped on ESX-1 related proteins reveal critical regions



Oren Tzfadia

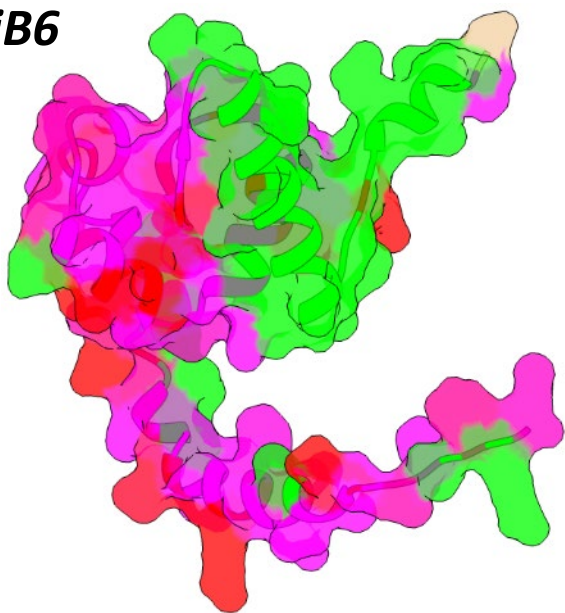


Axel Siroy

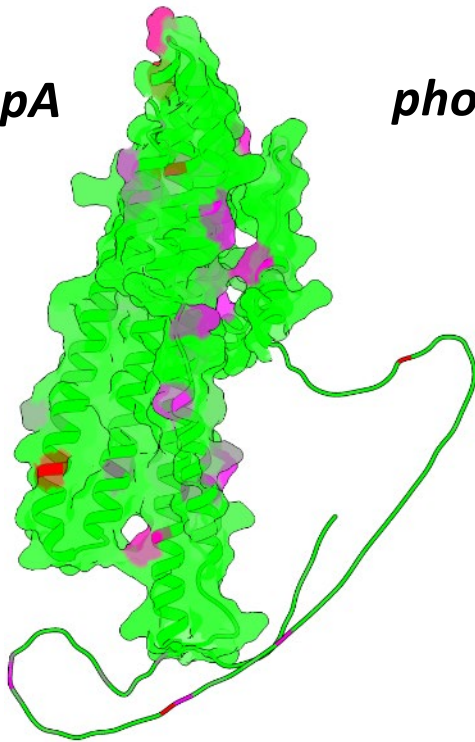


Roger Vargas

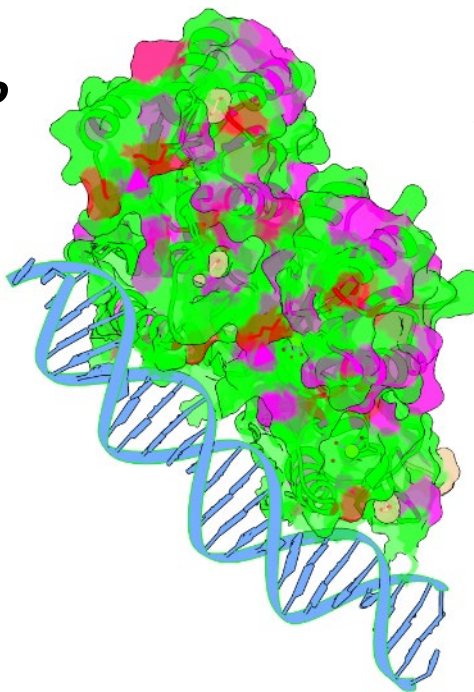
whiB6



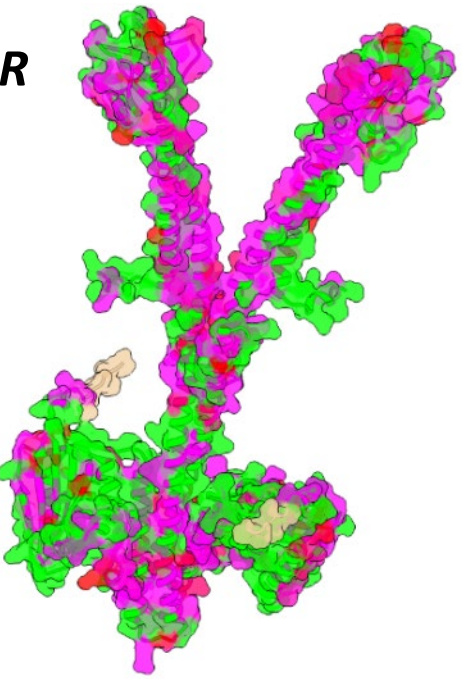
espA



phoP



phoR



→ Include in target selection for drugs and vaccines

Tzfadia et al, unpublished

Phenotype of ancestral lineages- L1, L5, L6

- Better adapted to specific populations, living at lower density
 - Transmit well to sympatric host, lower progression to disease
 - L5 + L6: Only sporadic transmission outside of West- and Central Africa
- Diagnostic gaps, caution on intrinsic resistance
- Clinically indistinguishable in an individual patient
- Different regulation of metabolism and virulence factors are incompletely understood

Short course on Clinical Decision-Making for Drug-Resistant TB (DR TB)



In the field of DR TB care, new molecular diagnostic tests, shorter treatment regimens and new drugs have been introduced recently. Clinicians require training in the use of these new diagnostic tests and in adequate and timely clinical decision-making.

INTERACTIVE TRAINING ON CLINICAL ASPECTS OF DR TB DIAGNOSIS AND CARE

WHAT WILL YOU LEARN?

- To define the problems with DR TB in your country in terms of occurrence, diagnosis and treatment, using available data;
- To assess harm and benefit of clinical decisions in the field of DR TB diagnosis and treatment;
- To formulate contextualized evidence-based recommendations for the prevention, diagnosis and treatment of DR TB for case studies from different contexts.



<https://edu.itg.be/courses/clinical-decision-making-for-drug-resistant-tuberculosis>

For clinicians with hands on experience treating TB

Deadline: ~30/11/2023

Thanks to



r2STOP

Research to STOP neglected tropical disease transmission.



This project is part of the EDCTP2 programme supported by the European Union



EDCTP



fwo



janssen



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