Population structure and evolution of the *Mycobacterium tuberculosis* complex

Pasteur Course, Shenzhen
17 October 2018

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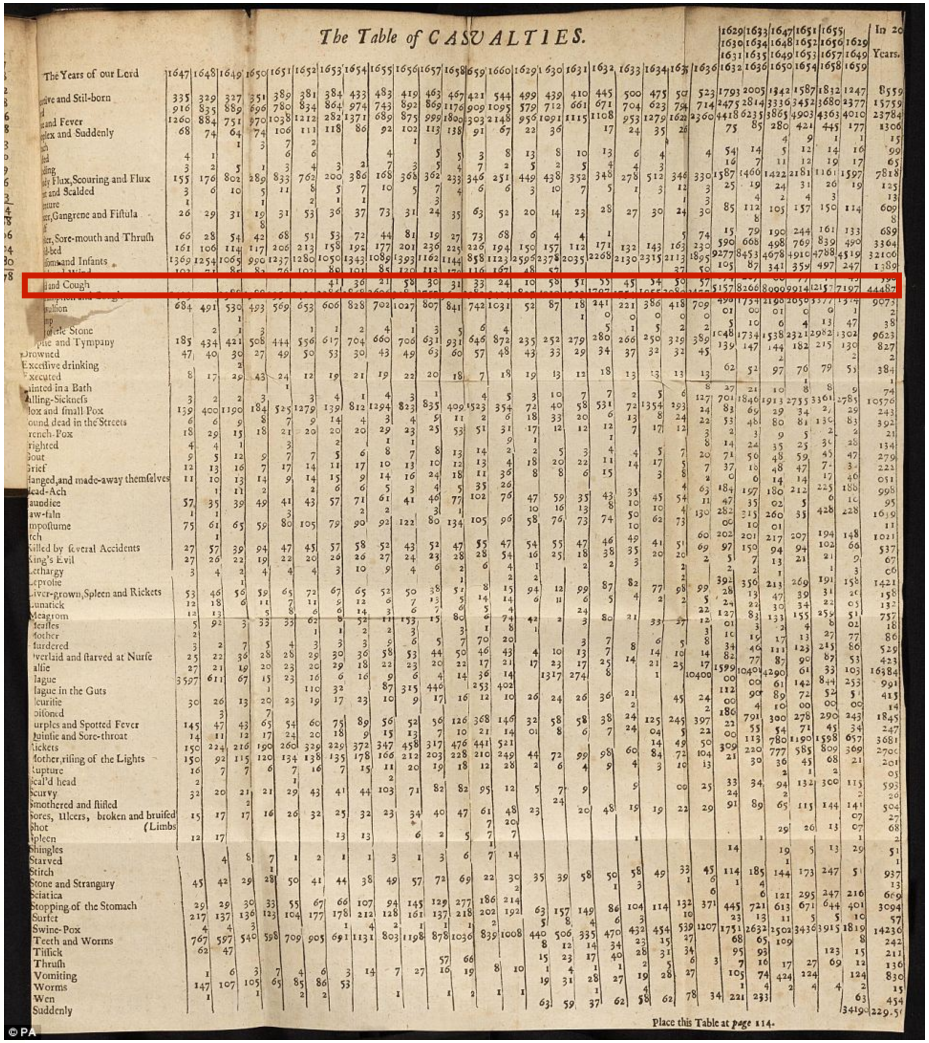


The Table of CASUALTIES.

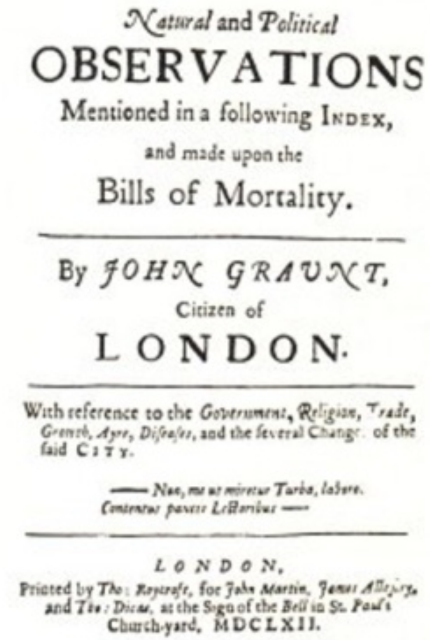
Place this Table at page 114.

© PA

Consumption (TB)
44,487 (19%)

Smallpox
10,576 (4.6%)

Plague
16,384 (7.13%)



Natural and *Political*
OBSERVATIONS
Mentioned in a following INDEX,
and made upon the
Bills of Mortality.

By *JOHN GRAUNT,*
Citizen of
LONDON.

With reference to the *Government, Religion, Trade, Growth, Ayre, Diseases,* and the several Changes of the said CITY.

—*Non, me ut miretur Turba, laboro, Contentus paucis Lectoribus* —

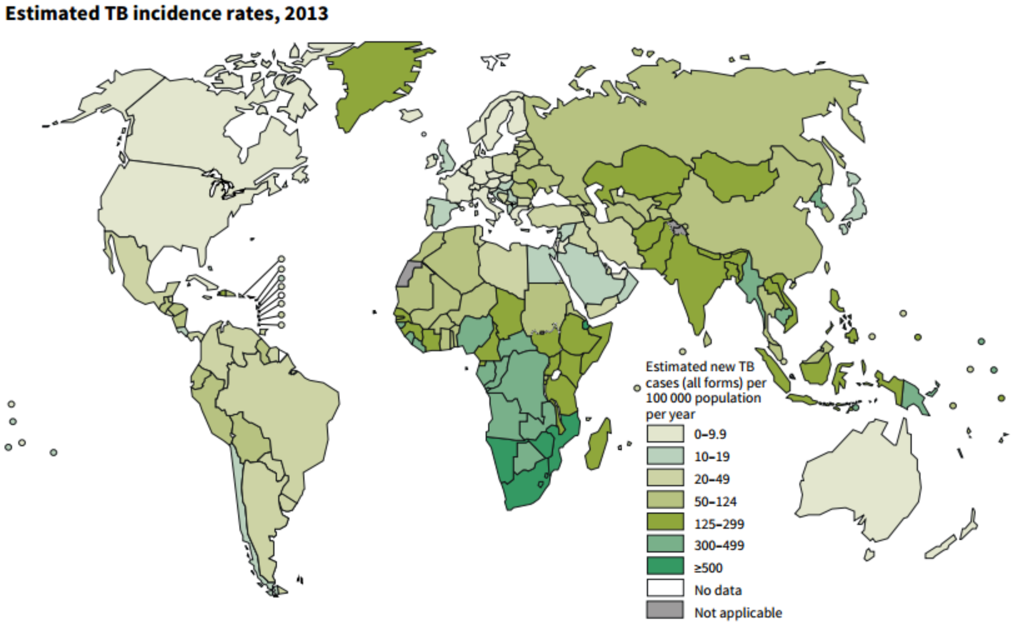
LONDON,
Printed by *Tho: Roycroft,* for *John Martin, James Allestry,* and *Tho: Dicas,* at the Sign of the Bell in St. Paul's Church-yard, MDCLXII.

Graunt´s bills of mortality for the city of London parishes for 20 years (229,590 deaths, 1629-1659 discontinued)

GENERALITAT VALENCIANA

And in the present…

Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana


Estimated TB incidence rates, 2013

WHO Global Tuberculosis Report 2014

TB facts

More than 1.5 million deaths per year

Around 9 million new cases

One third of the global population infected

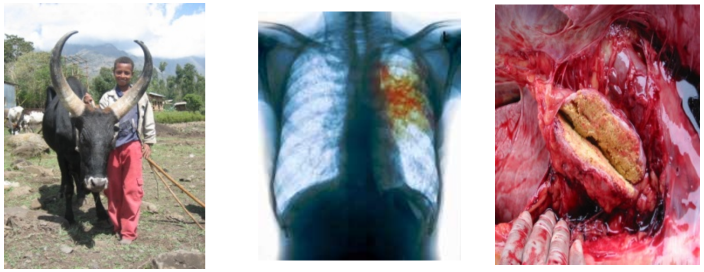
Drug resistant cases reported in every country

€5.8 billion economic burden to EU per year

Bovine TB facts

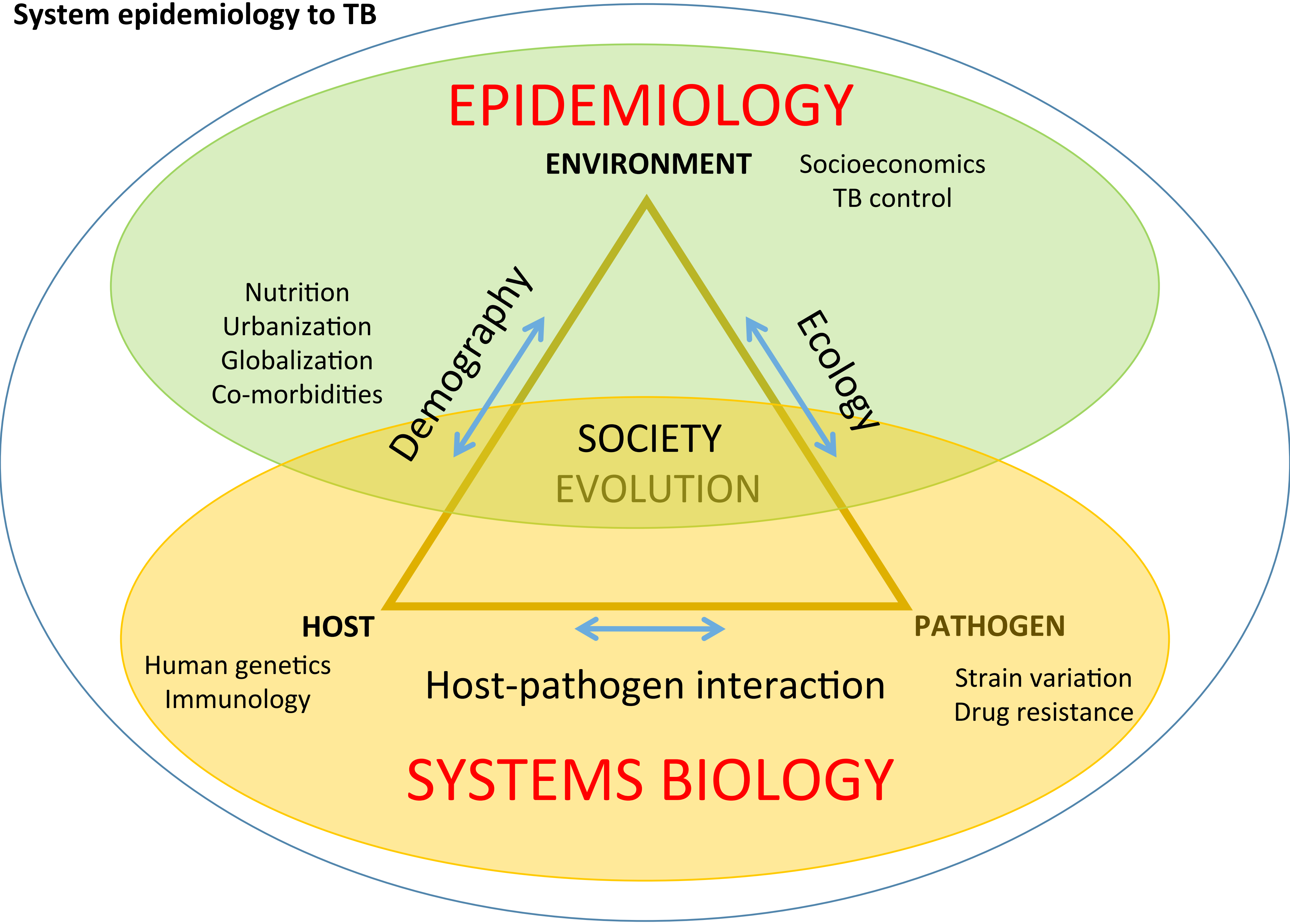
Unknown human burden: est. 1.4-2.8% human TB cases

€3 billion loses to global agriculture



CHAT
Community for Host pathogen diversity Awareness in Tuberculosis

System epidemiology to TB



EPIDEMIOLOGY

ENVIRONMENT

Socioeconomics
TB control

Nutrition
Urbanization
Globalization
Co-morbidities

Demography

Ecology

SOCIETY

EVOLUTION

HOST

Human genetics
Immunology

Host-pathogen interaction

PATHOGEN

Strain variation
Drug resistance

SYSTEMS BIOLOGY

Comas I. & Gagneux S. PloS Pathogens (2009) and Comas I. & Gagneux S. Trends in Microbiology (2011)

The *M. tuberculosis* complex:

Human versus Animal TB

M. africanum

M. tuberculosis

M. bovis

***M. bovis* hyrax**

M. pinnipedii

M. microti

M. bovis* subsp. *caprae

***M. bovis* antelope**

The two faces of a pathogen

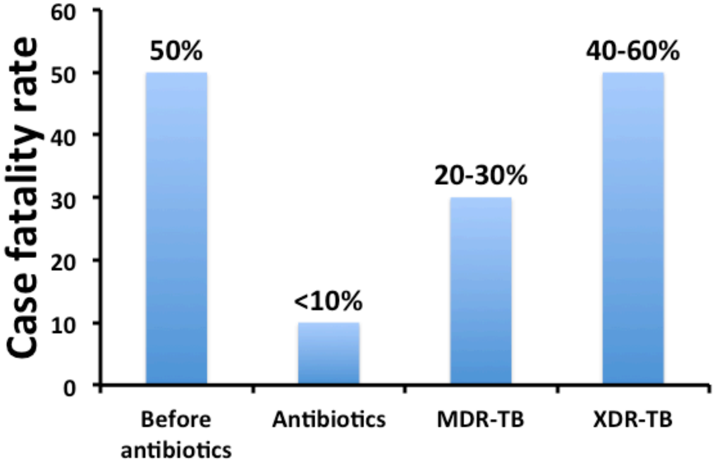
Obligate human pathogen

9 million new cases/ 1.5 million deaths

M. africanum

M. tuberculosis

Kills up to 50% if untreated



One third latently infected

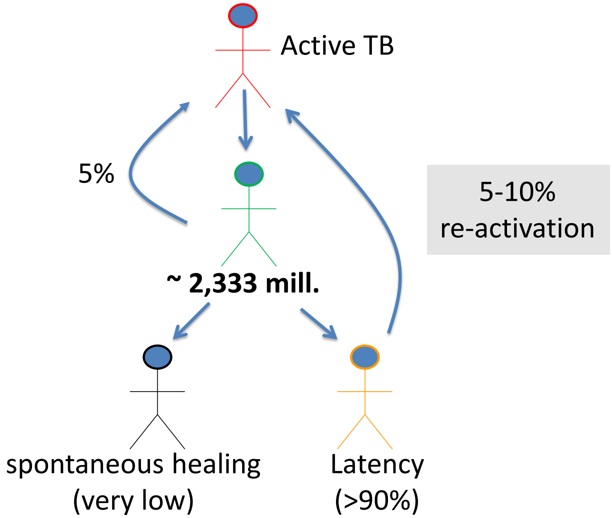
Active TB

9 million new cases

Latent TB

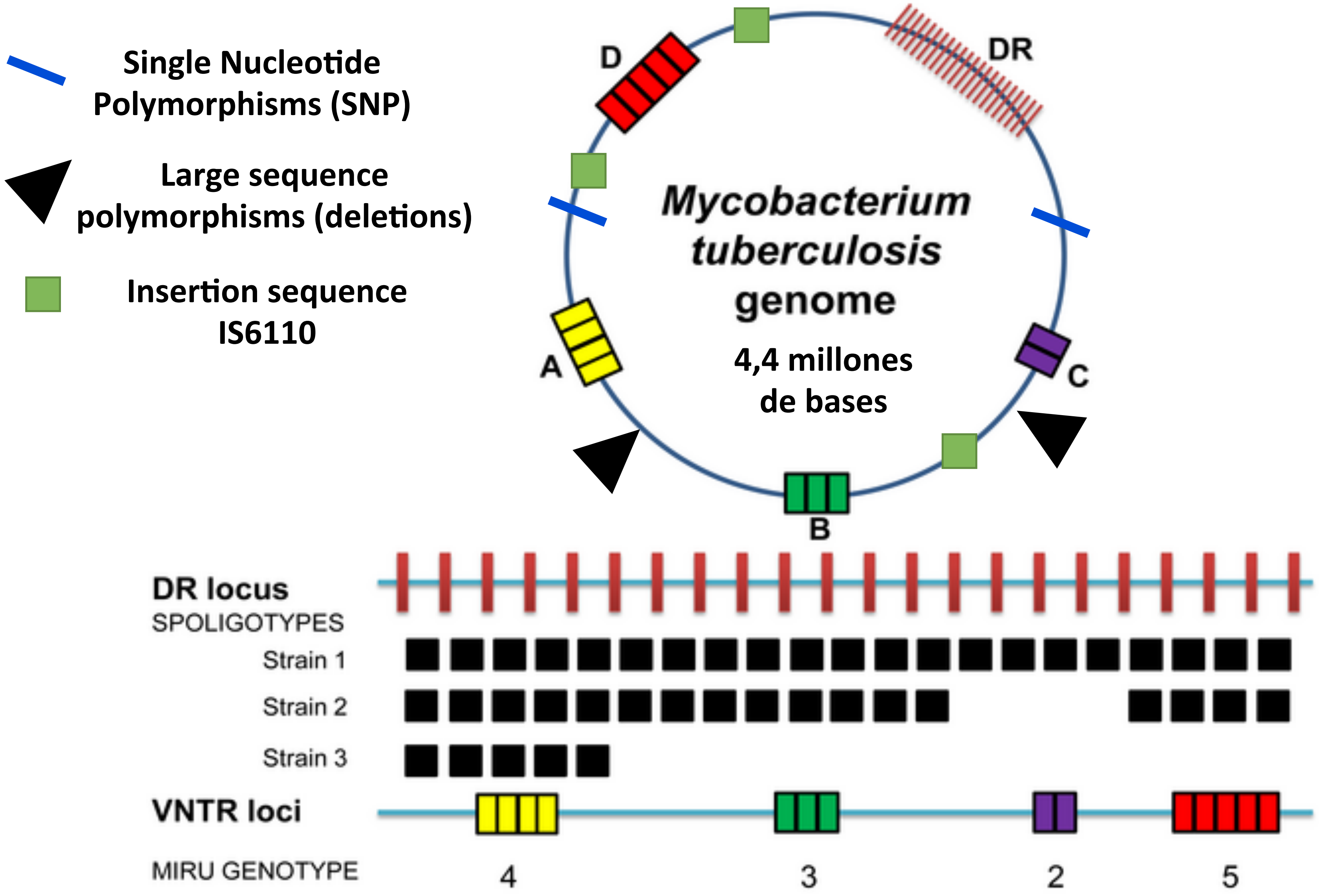
2.3 billion

Only 10% progress to active TB



THIS TALK

- Genome sequencing technologies
- Global diversity of the MTBC
- Evolutionary forces and selective pressures in the MTBC
- Diversification of MTBC at a global scale
- Diversification during infection and transmission



— Single Nucleotide Polymorphisms (SNP)

◀ Large sequence polymorphisms (deletions)

🟩 Insertion sequence IS6110

Comas et al. PloS One 2009 (modified)

MIRU-VNTR
RFLP

MOLECULAR
EPIDEMIOLOGY

+

SPOLIGO
DELETIONS
SNP-TYPING

Population structure
Evolution

GENOME

How to interrogate?

Short-read

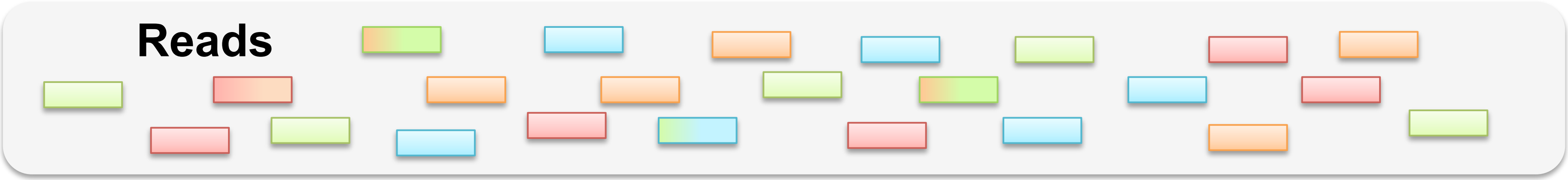
Illumina

ANALYSIS PIPELINE WILL DEPEND ON TECHNOLOGY

Long-read

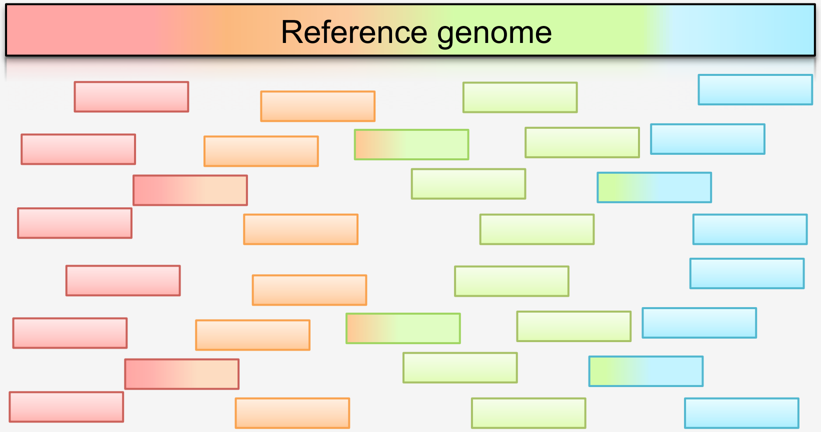
PacBio
Nanopore

Differences between mapping and *de novo* assembly



Mapping

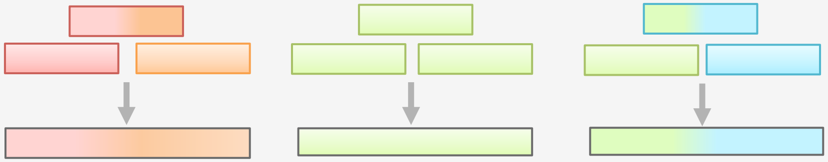
Compare with a reference genome



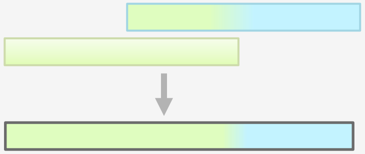
SAM

De novo assembly

Find overlaps between reads



Assembly overlaps into contigs



Assembly contigs into scaffolds

FASTA/Q

Short-read

Illumina

Pros:
Paired-end reads
Up to 2x300pb
Low error rate
High multiplexing

Cons:
Limited structural information
GC-content bias
Reference mapping

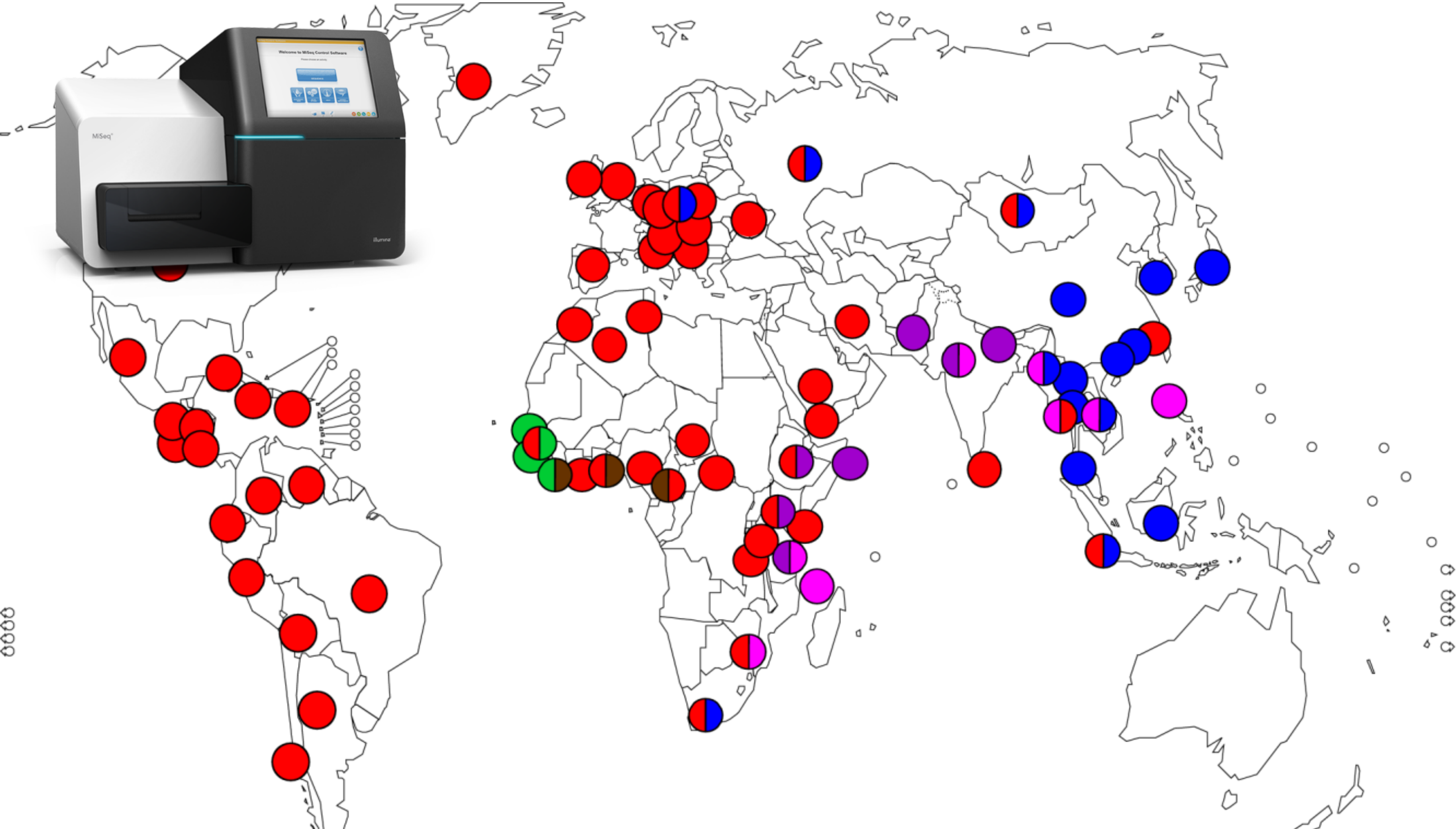
Long-read

PacBio
Nanopore

Pros:
High structural information
'Real time' sequencing
'No' GC-bias
De-novo assembly

Cons:
high base errors ~11-15%
Accuracy
Low multiplexing

MTBC global diversity



Comas et al. PloS Pathogens (2009)

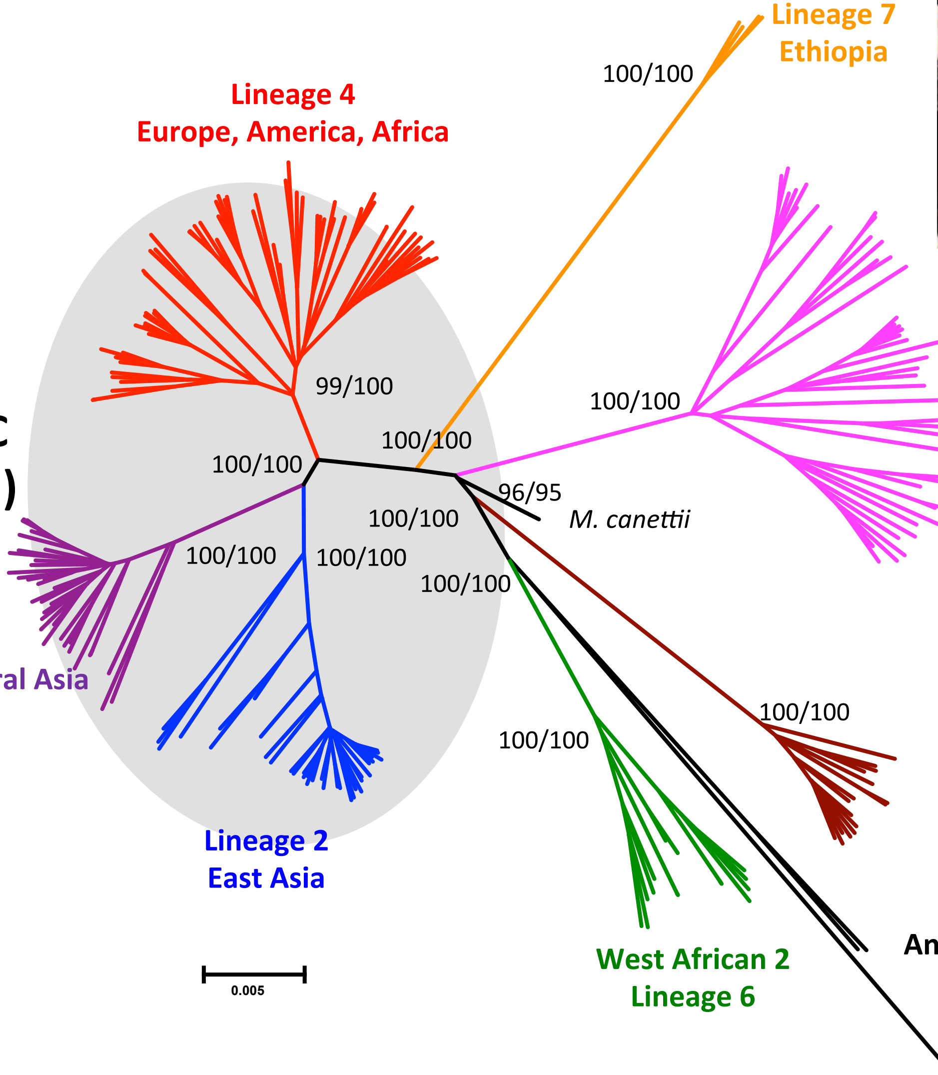
A genome-based MTBC phylogeny

220 clinical strains

34,167 SNPs

Modern MTBC (TbD1 deleted)

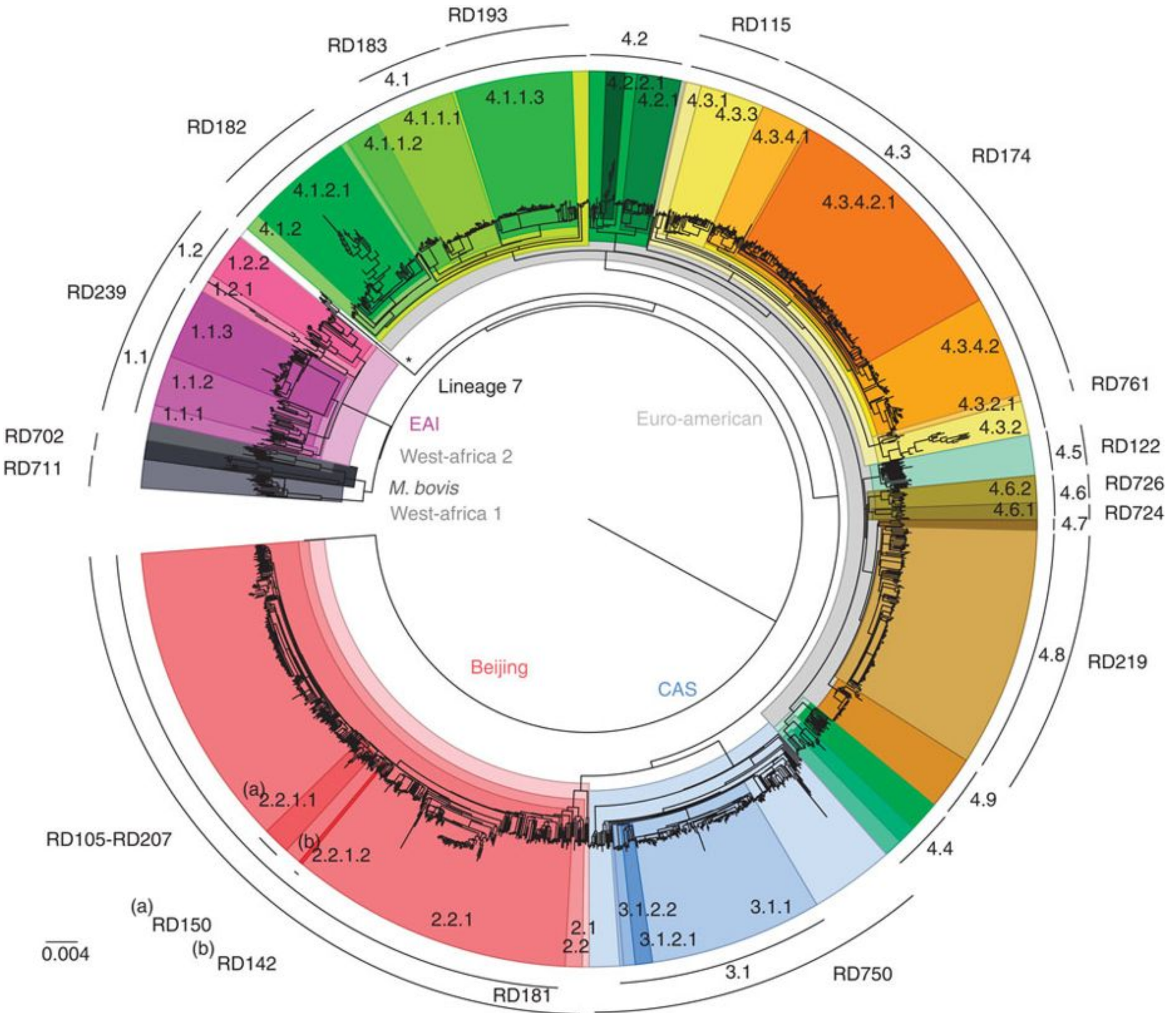
Brosch 2002



Ethiopia highlands

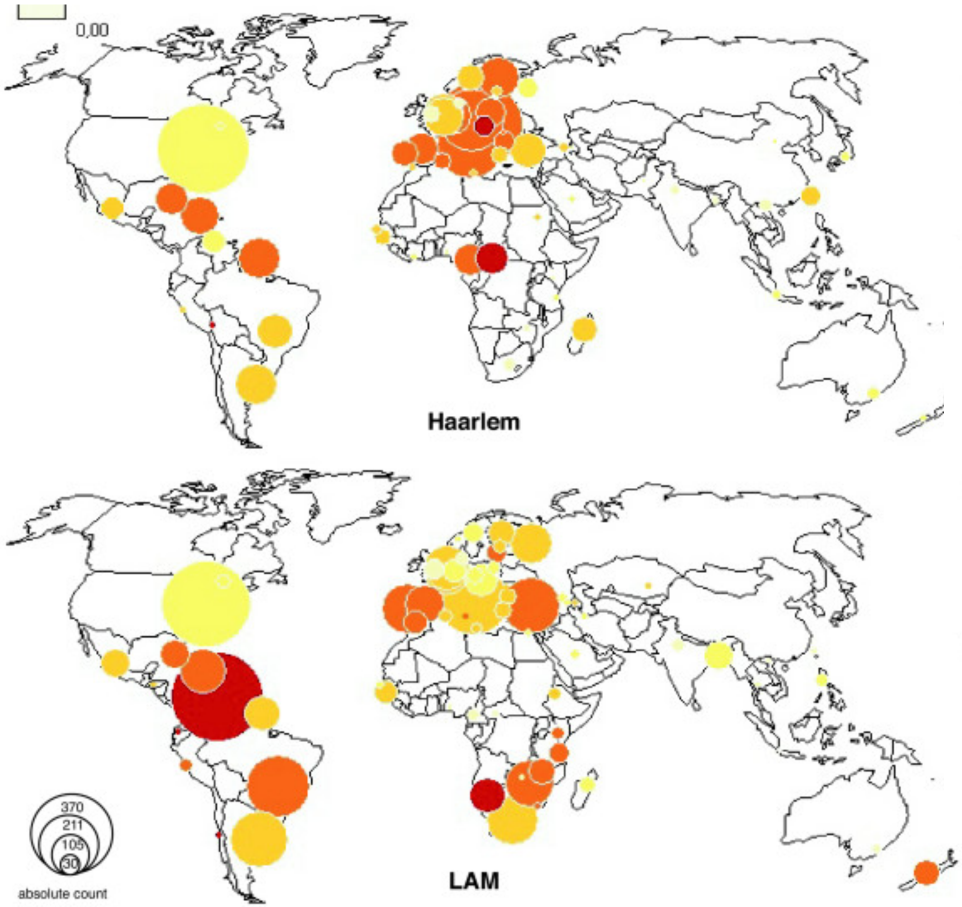
Comas et al. Nat. Genet. 2013

USING GENOME PHYLOGENIES TO DEFINE A BARCODE FOR MTBC

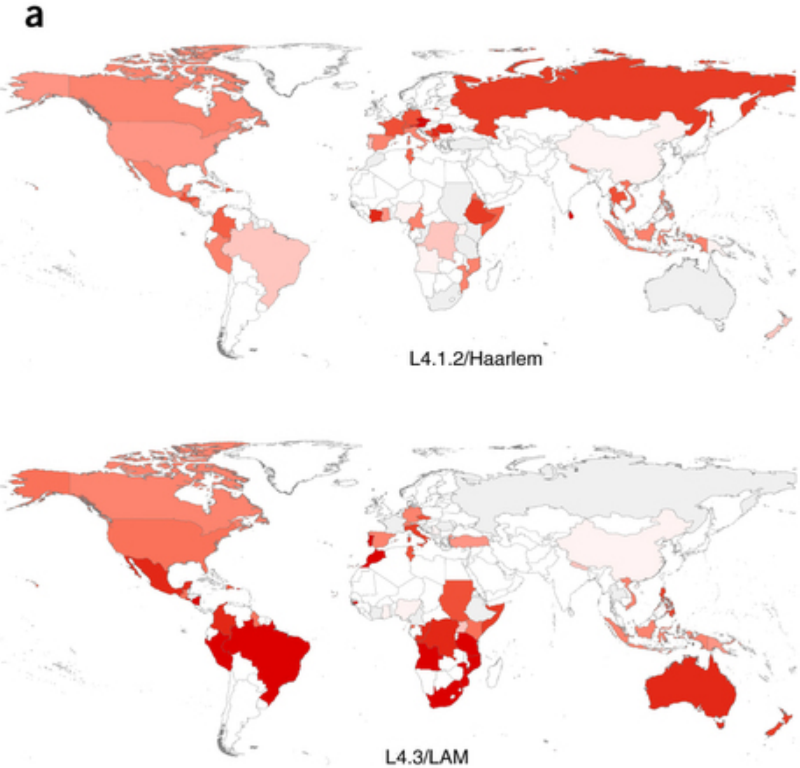


Coll et al. Nat Com 2014

From global to local: from spoligo to genomes

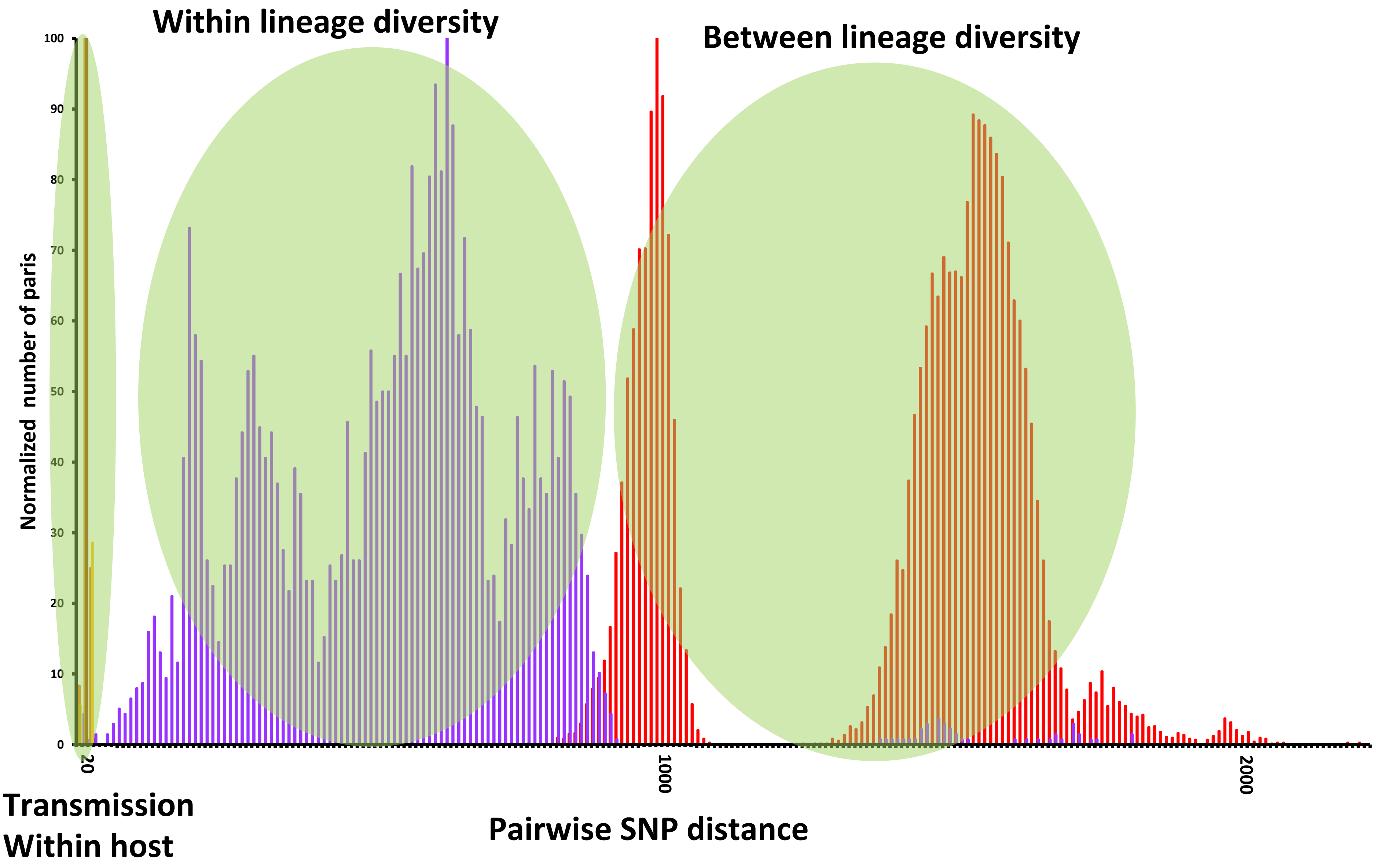


Brudey et al. BMC Microbiology 2006

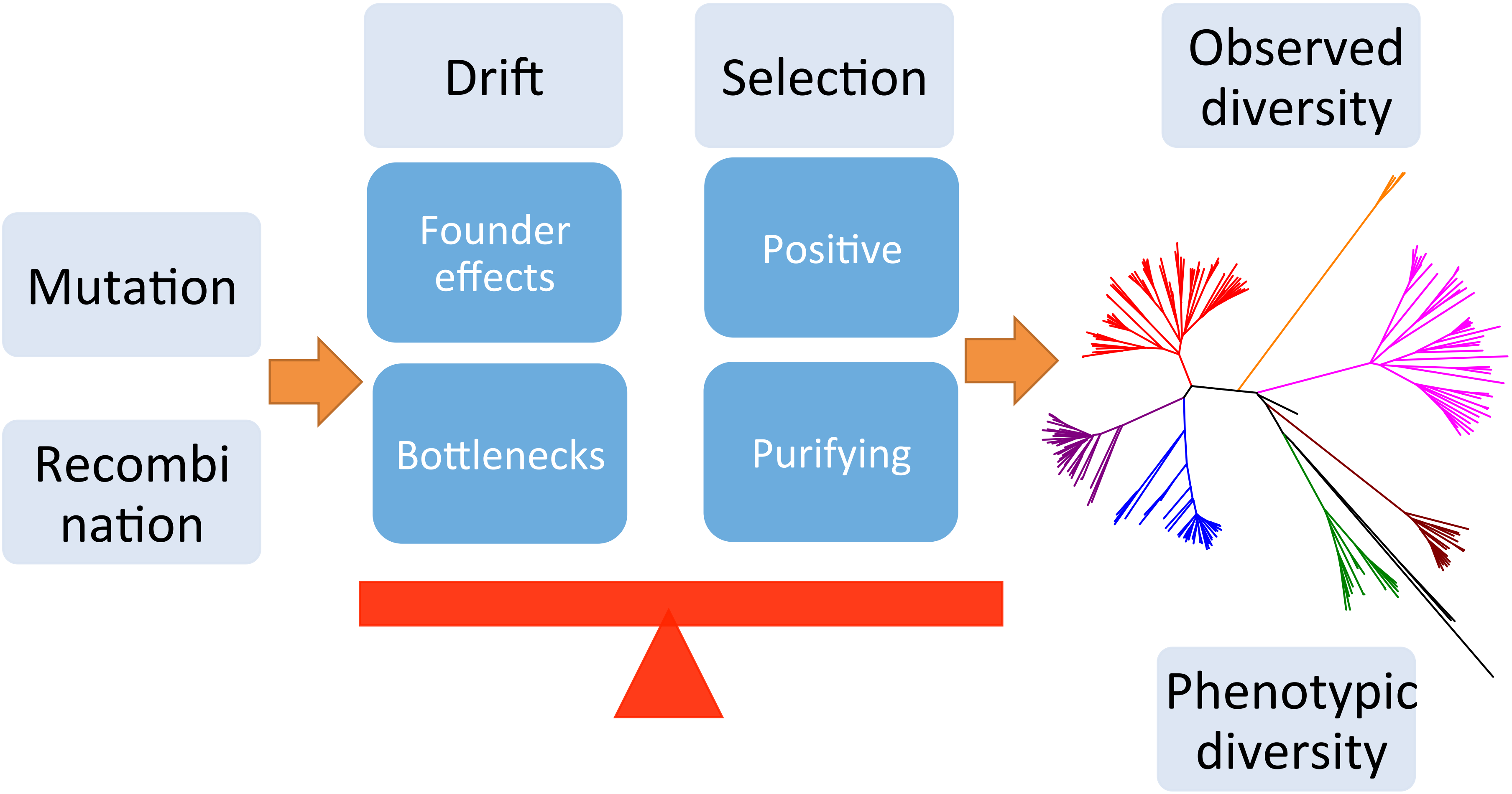


Stucki, Brites et al. Nat Genet 2016

Understanding TB genomic diversity: from millennia to minutes



Observed diversity is a balance of evolutionary forces



Mutation

Recombination

Drift

Founder effects

Bottlenecks

Selection

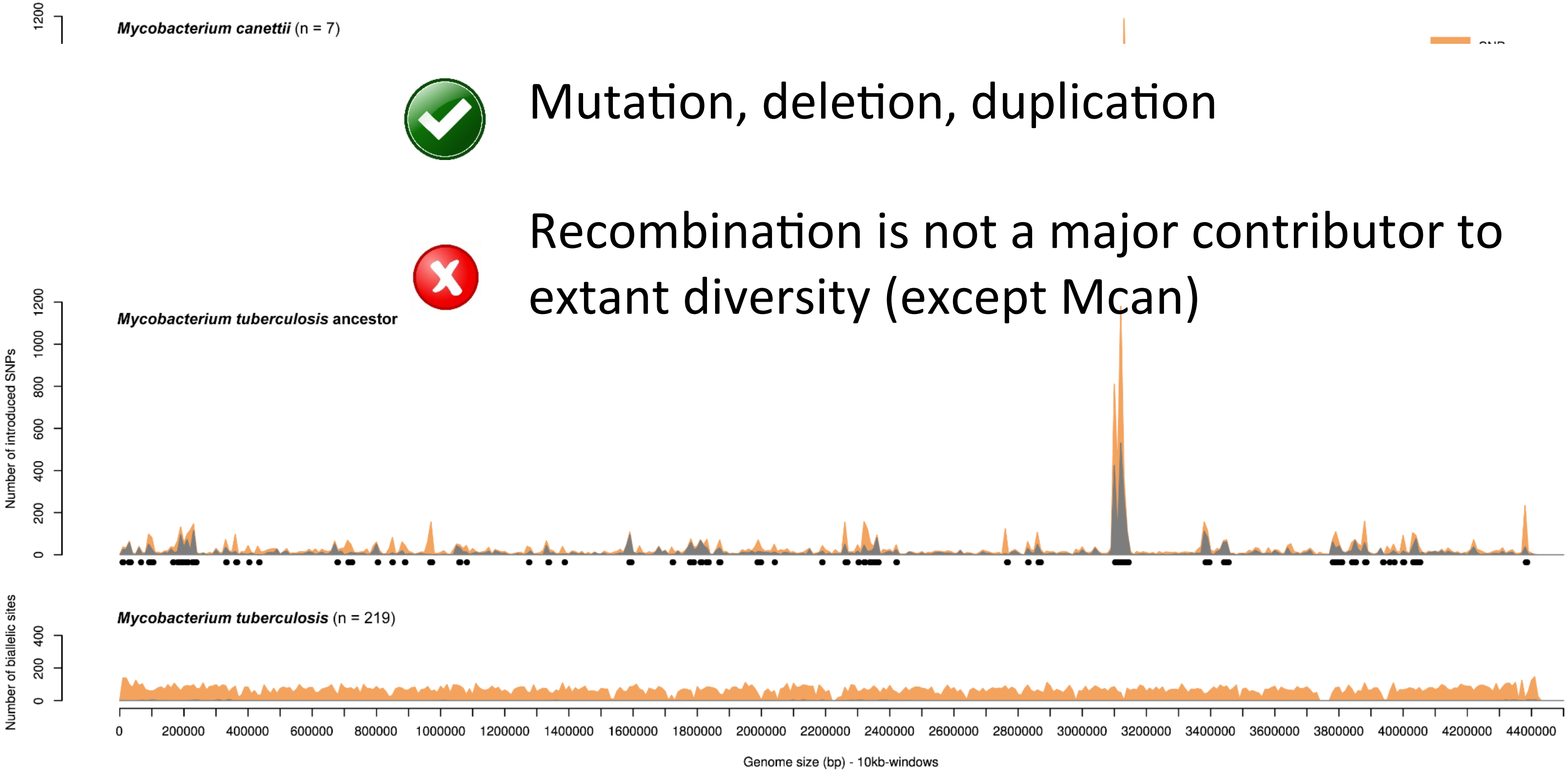
Positive

Purifying

Observed diversity

Phenotypic diversity

Evolutionary forces in MTBC



✓ Mutation, deletion, duplication

✗ Recombination is not a major contributor to extant diversity (except Mcan)

Chiner-Oms A., Sánchez-Busó L. et al. (under review)

Observed diversity is a balance of evolutionary forces

Mutation

~~Recombination~~

Drift

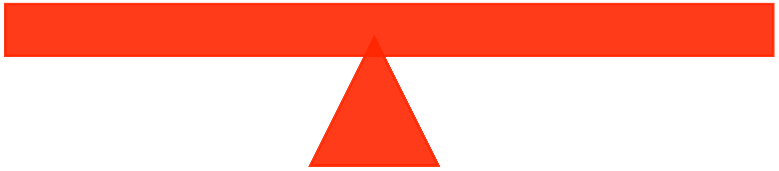
Founder effects

Bottlenecks

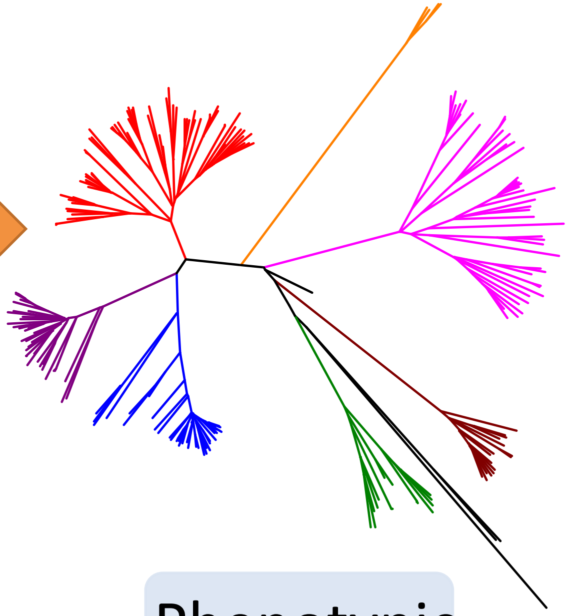
Selection

Positive

Purifying

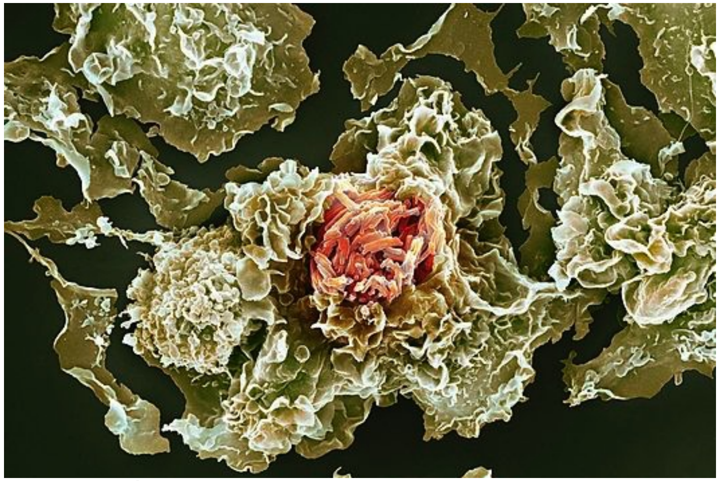


Observed diversity

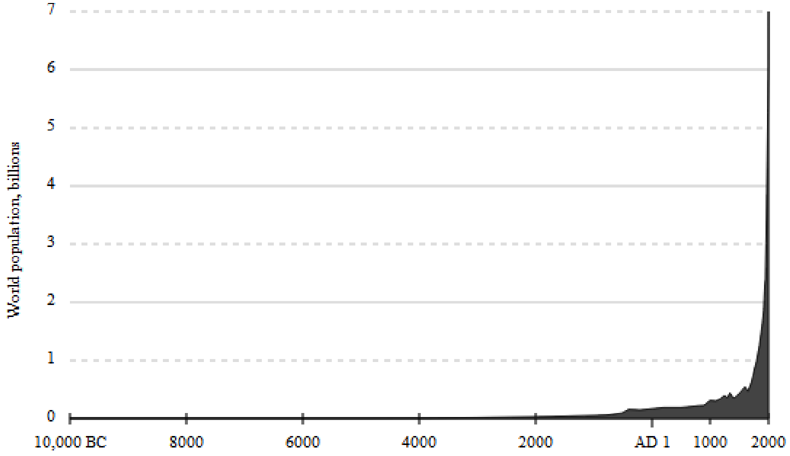


Phenotypic diversity

Old and new selective pressure in MTBC evolution



Immune system

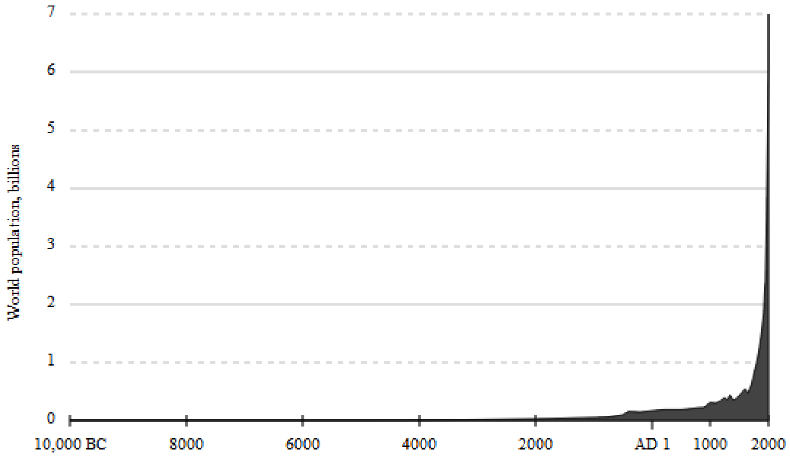


Human population

Modern epidemics

Antibiotics

Old and new selective pressure in MTBC evolution

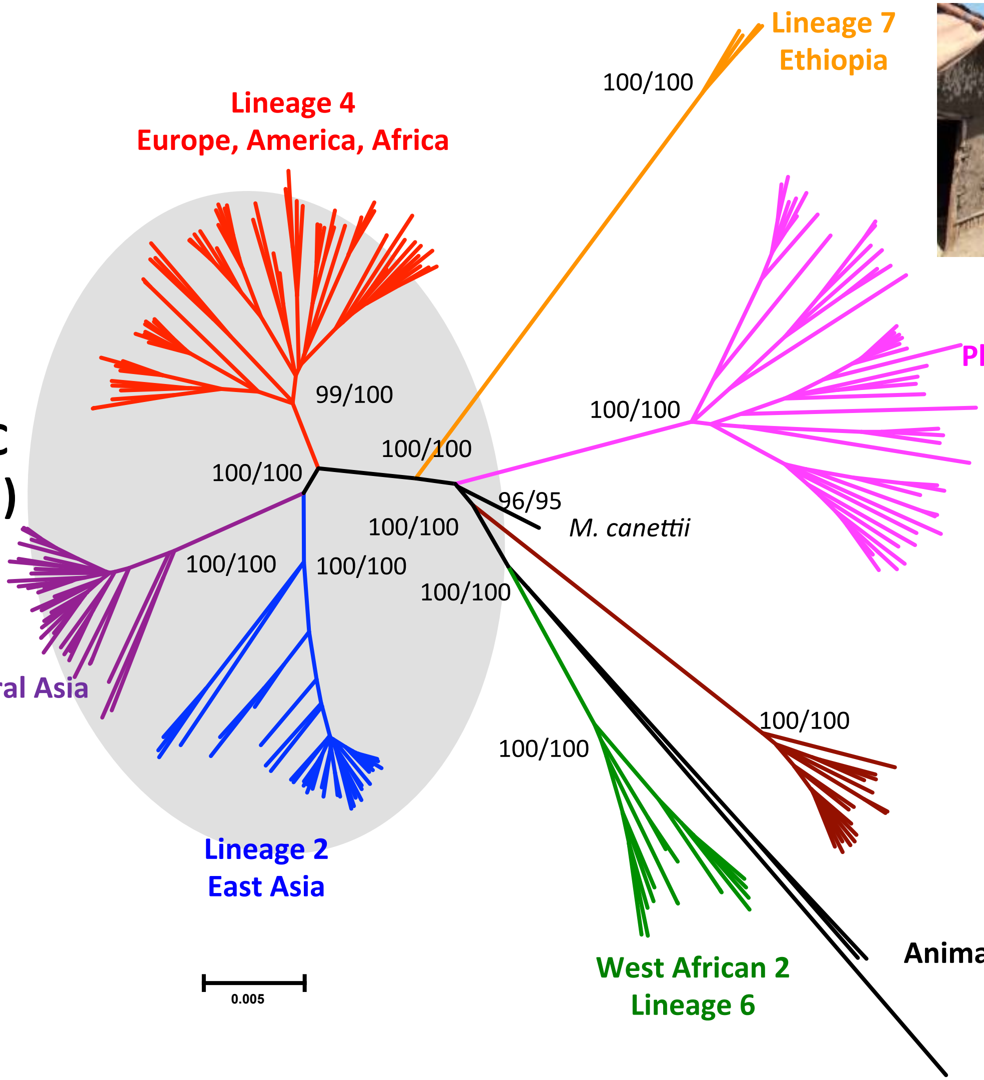


Human population

A genome-based MTBC phylogeny

220 clinical strains

34,167 SNPs

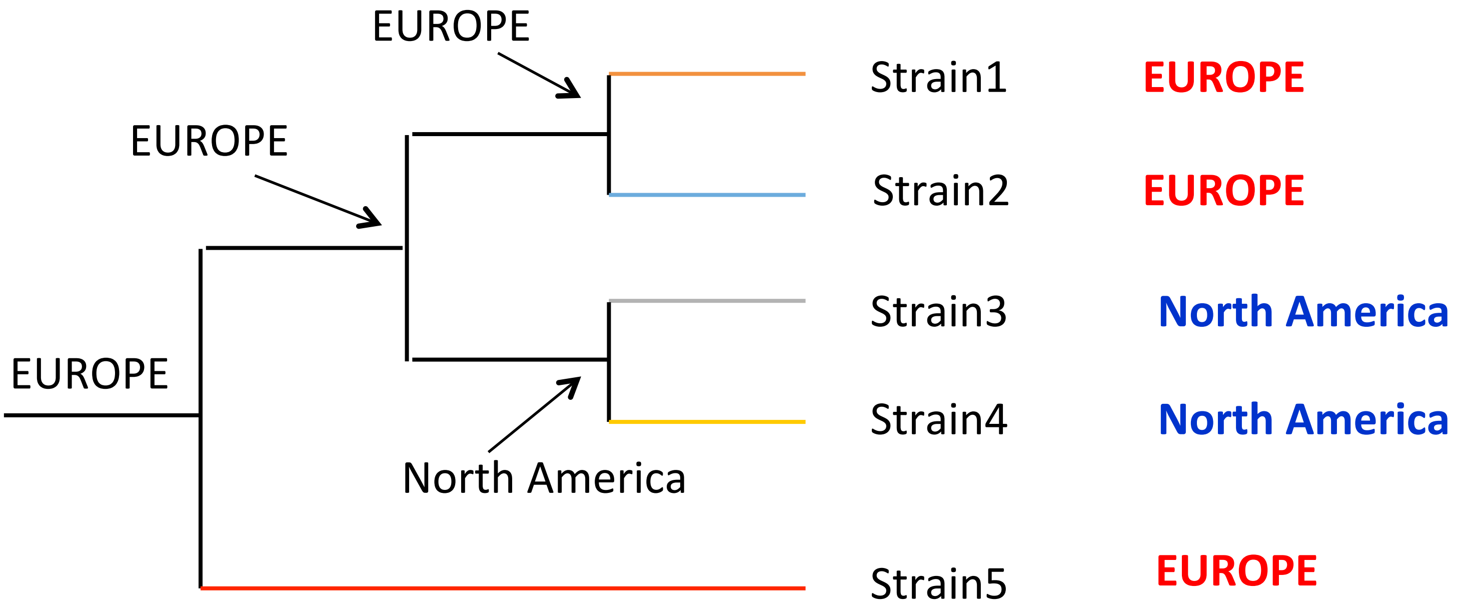


Modern MTBC (TbD1 deleted)
Brosch 2002

Ethiopia highlands

Comas et al. Nat. Genet. 2013

Phylogeography can help to determine the origin of the disease



EUROPE

EUROPE

EUROPE

North America

Strain1 **EUROPE**

Strain2 **EUROPE**

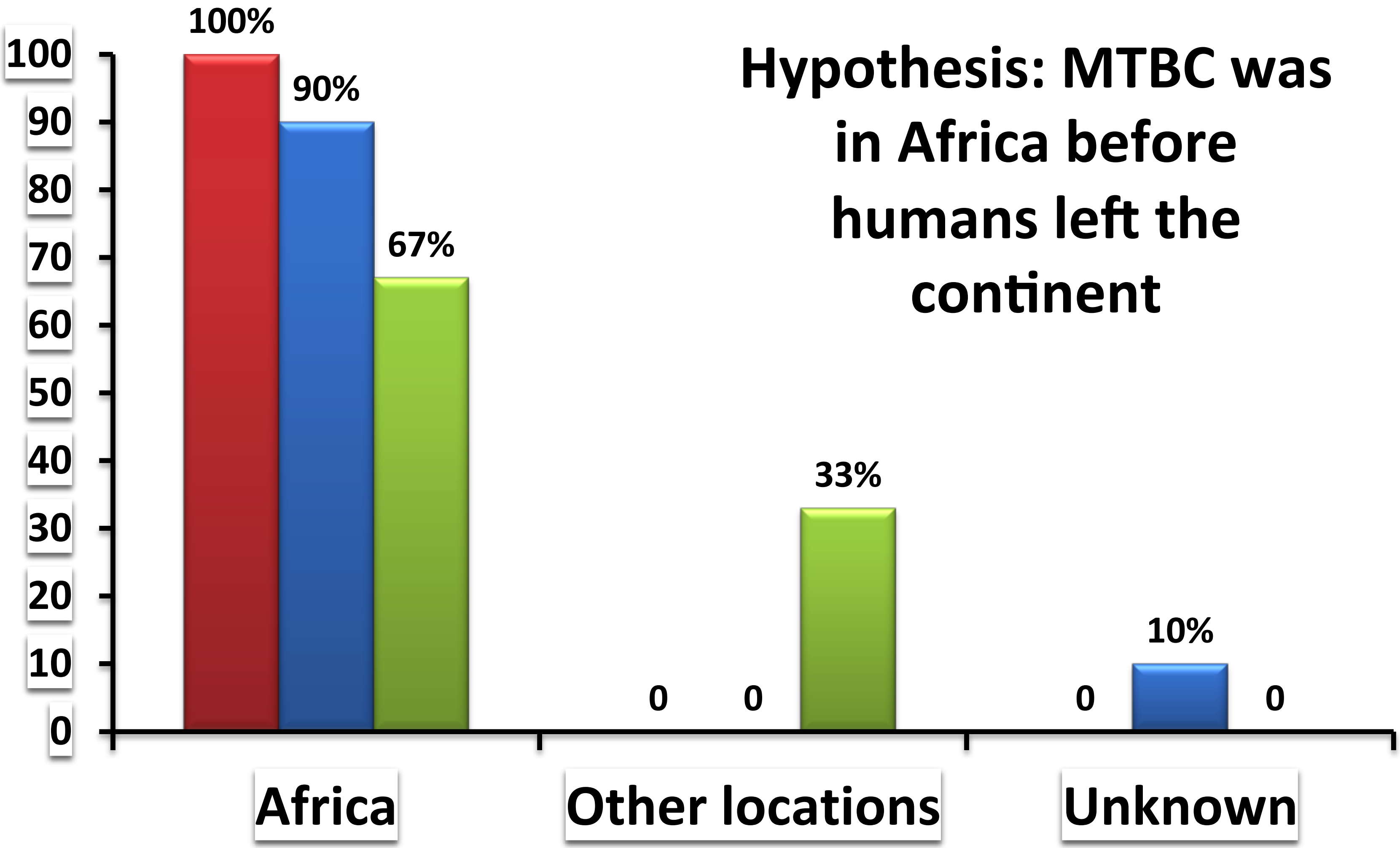
Strain3 **North America**

Strain4 **North America**

Strain5 **EUROPE**

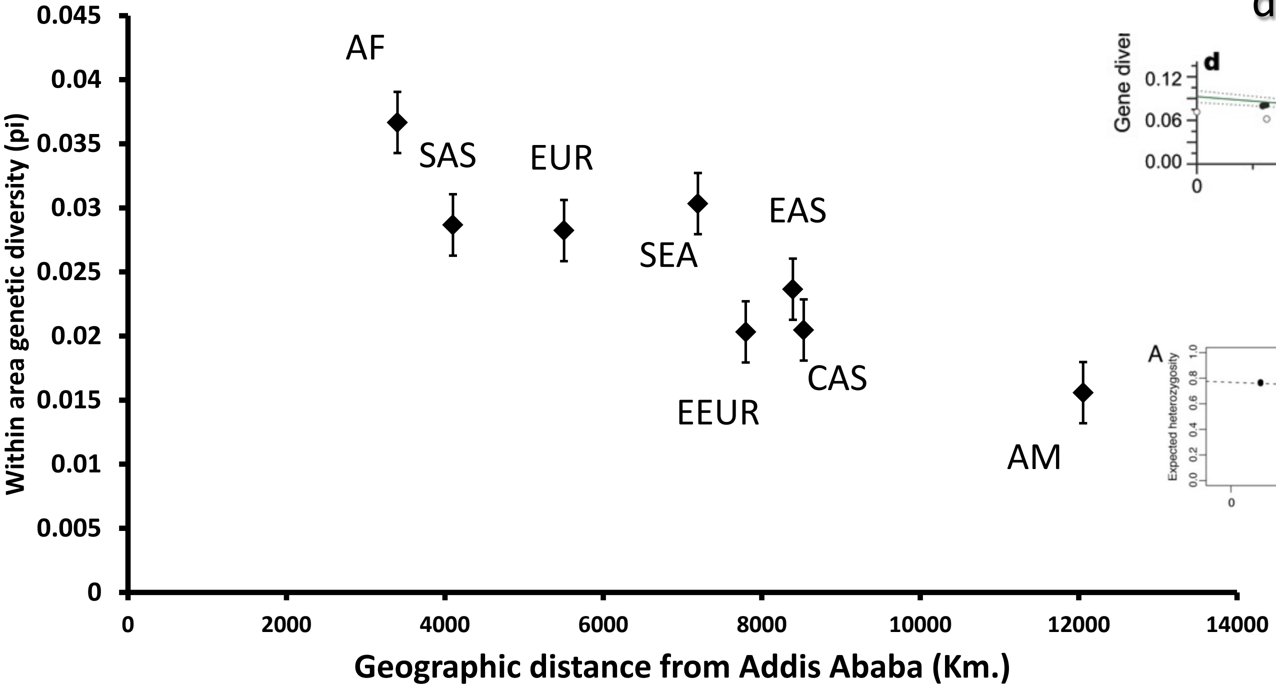
There are several maximum likelihood, maximum parsimony and bayesian approximations for phylogeographic analyses

Three different phylogeographic analyses confirm <u>Africa as the origin of the disease</u>

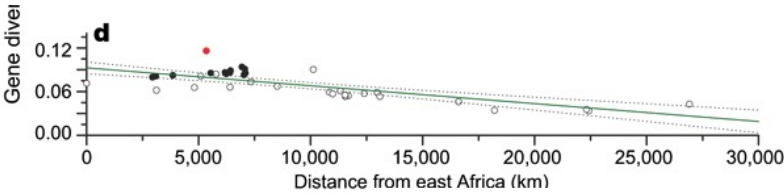


Hypothesis: MTBC was in Africa before humans left the continent

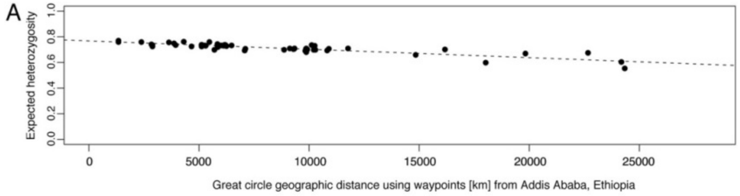
Horn of Africa is the most likely origin of MTBC



Decreasing diversity with geographic distance from East Africa



Human

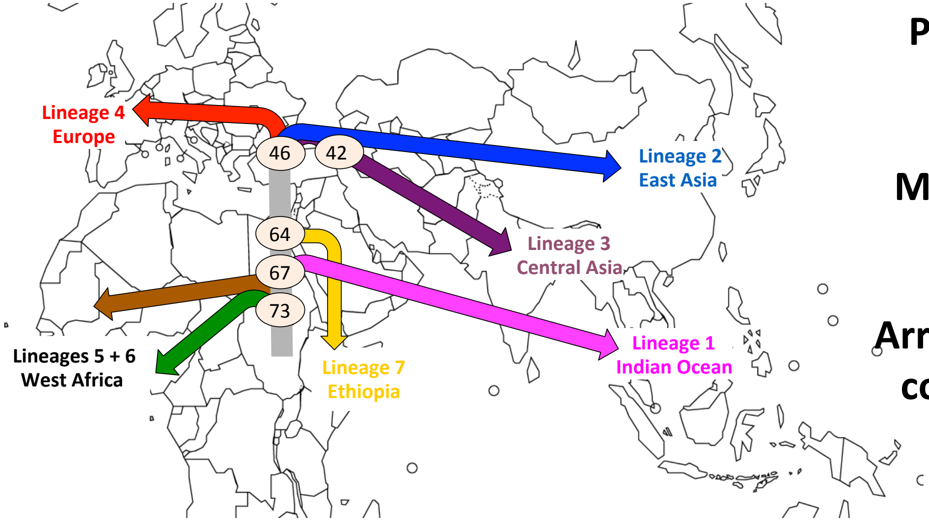


H. pylori

Geographic distance from East Africa explains 71% of the MTBC diversity

Comas, Young, Aseffa, Gagneux, Berg (Current Biology 2015)

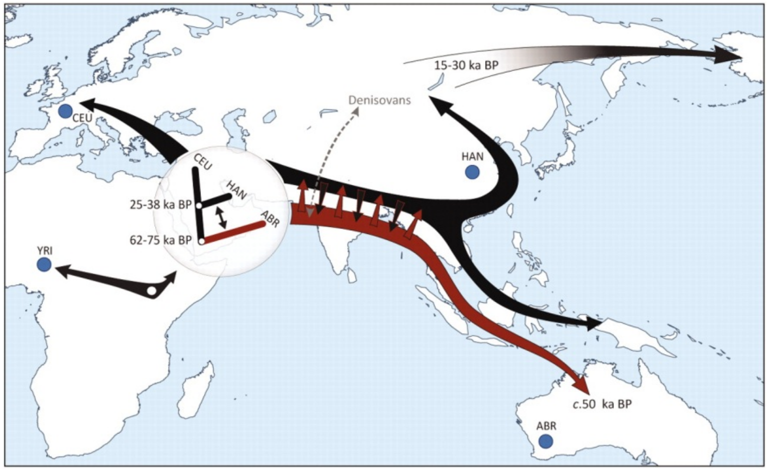
TB is 70 thousand years old and mimics human migrations



Present day TB clinical strains share a common ancestor 70 K years ago

Migrated Out of Africa with humans in two waves (around 65K years ago)

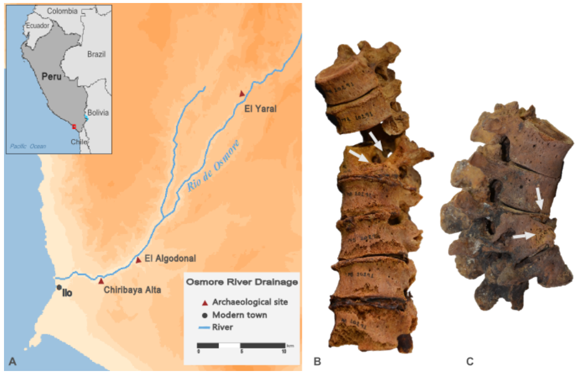
Arrival to Europe (~40 Kya) and East Asia (~30 Kya) coincides with the first archeological findings of human presence



Rasmussen, Science 2011

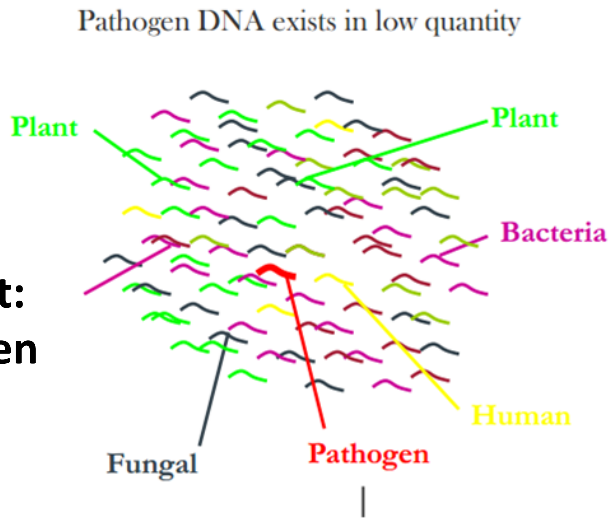
The Genographic project

Ancient MTBC DNA recovered from peruvian mummies



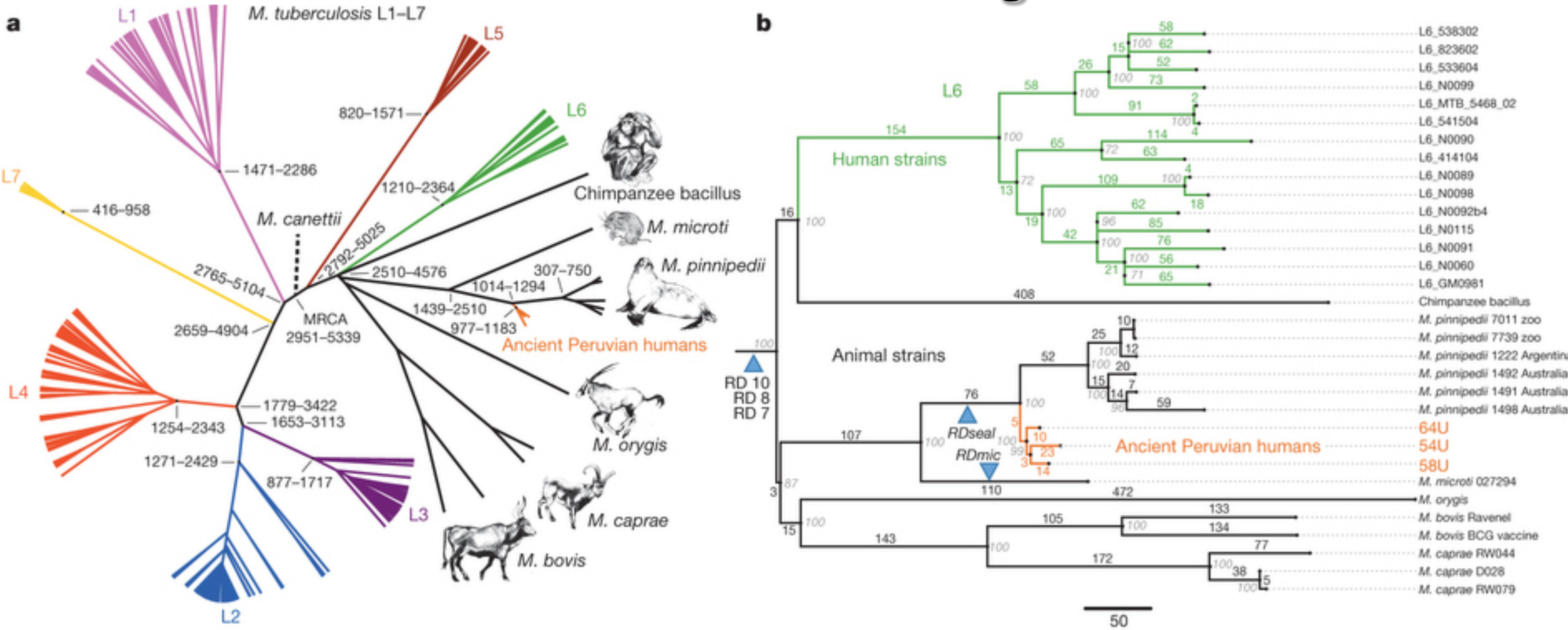
Peruvian mummies with bone scars compatible with TB (1,000 years old)

Targeted DNA enrichment: fishing for ancient pathogen DNA



Bos et al. Nature 2014

Ancient DNA reveals seals TB as the closest extant bacilli, doubts about timing

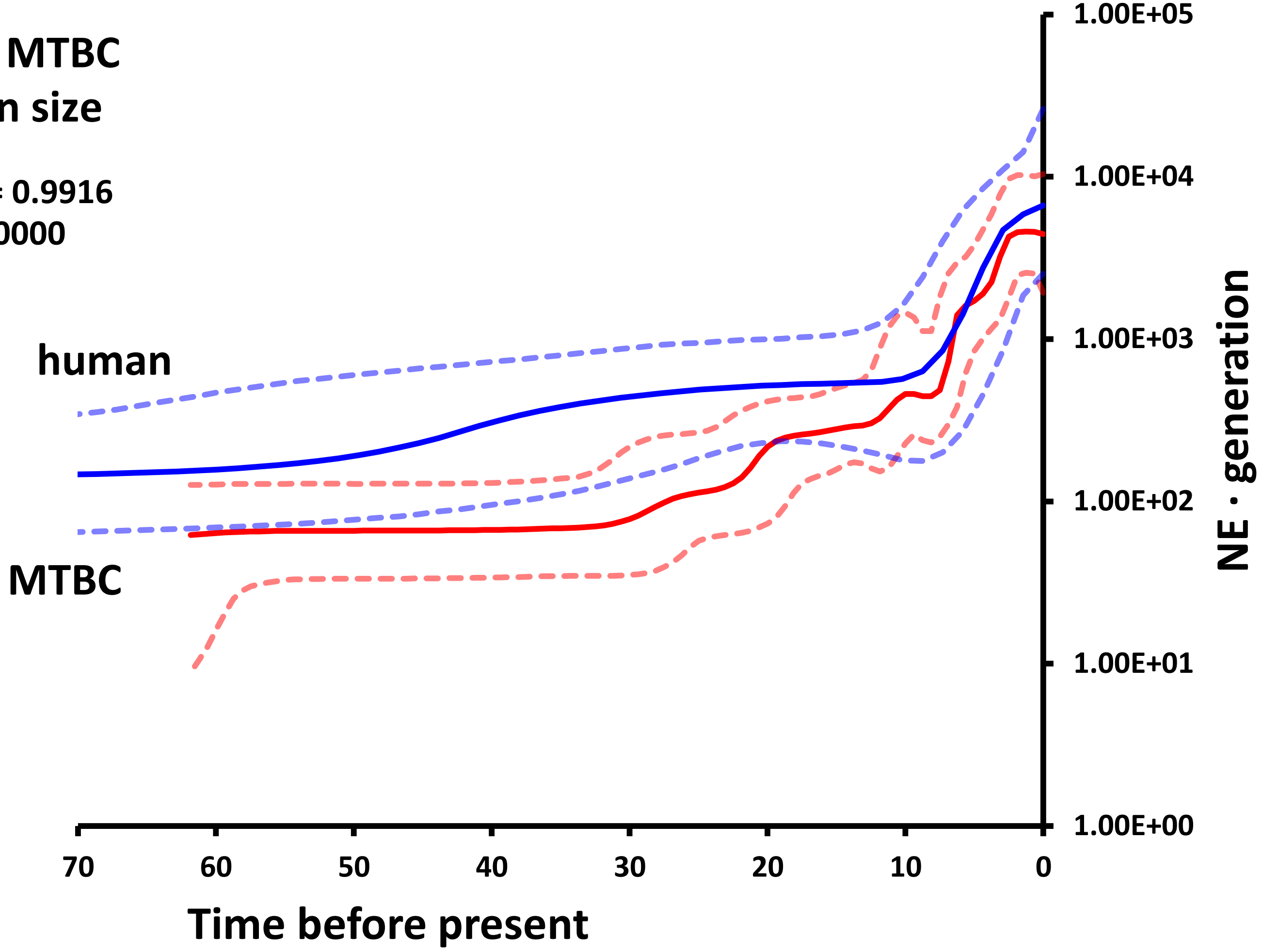


Bos et al. Nature 2014

Human-driven expansions of the MTBC

Human vs. MTBC population size

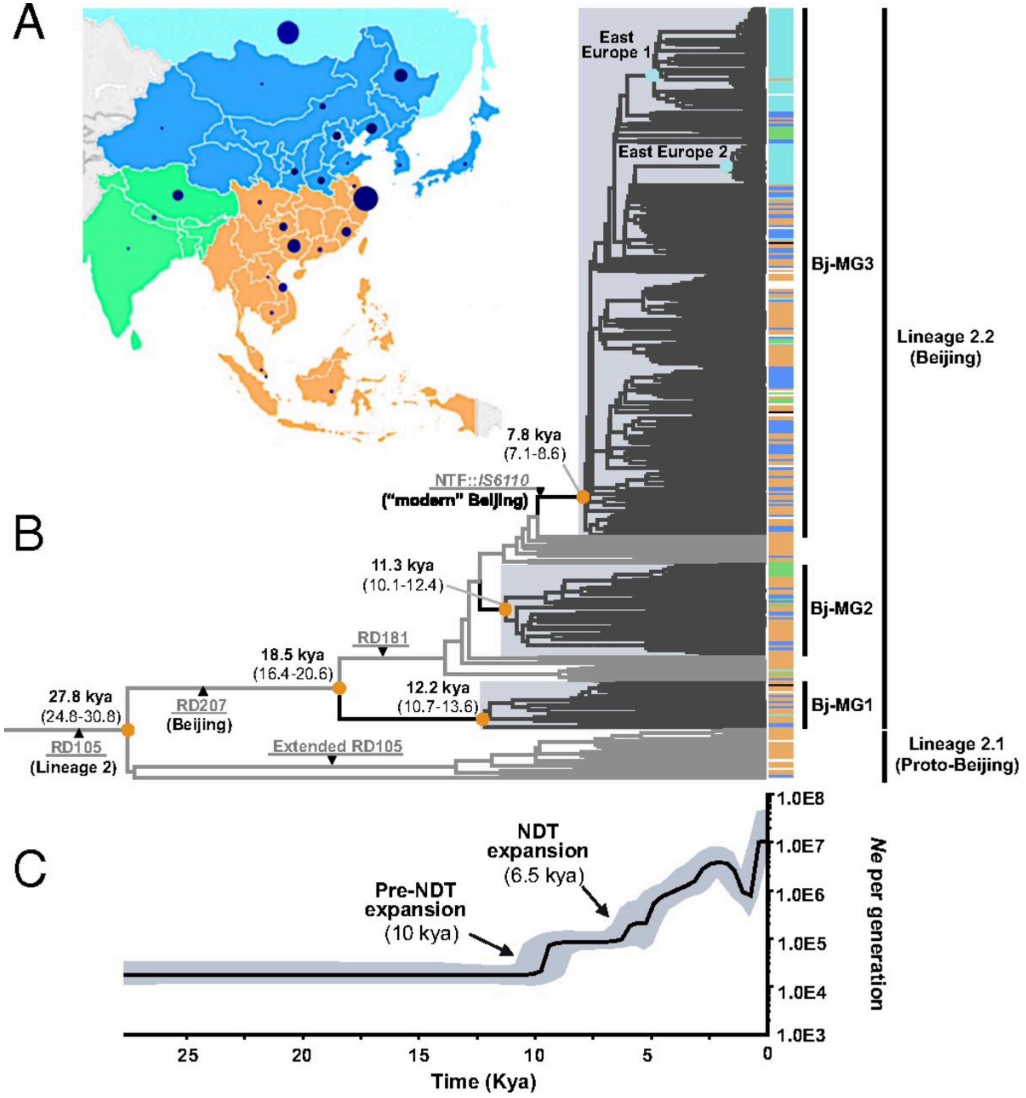
Spearman R = 0.9916
P-value 0.0000

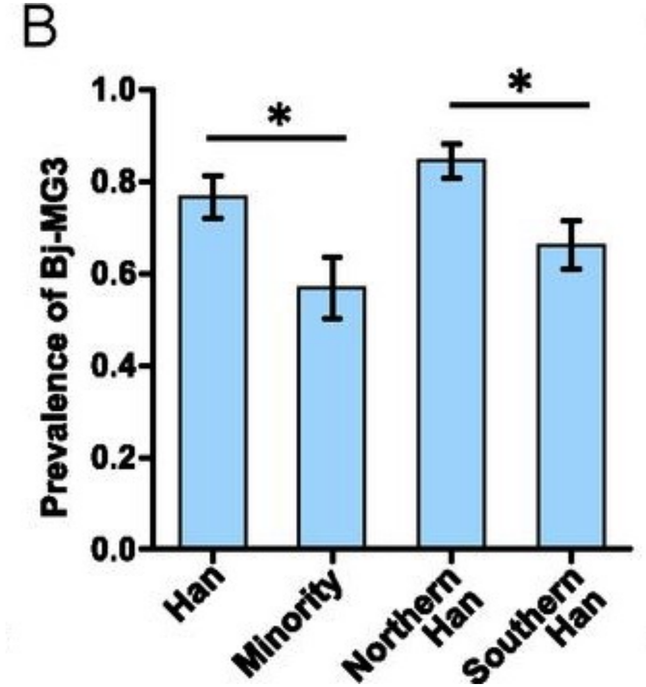


MTBC has been able to adapt to changing human densities

Comas et al. Nat. Genet. 2013

Local expansions driven by biology and historical contingency

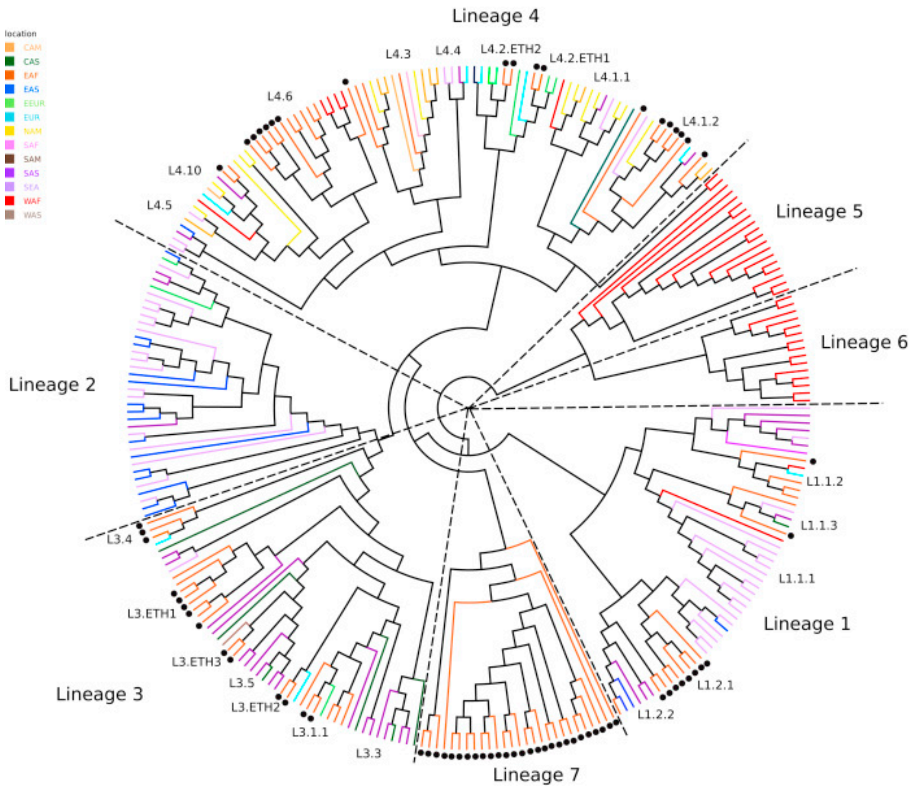




Luo et al. PNAS 2015, Liu et al. Nat Ecol Evol (accepted)

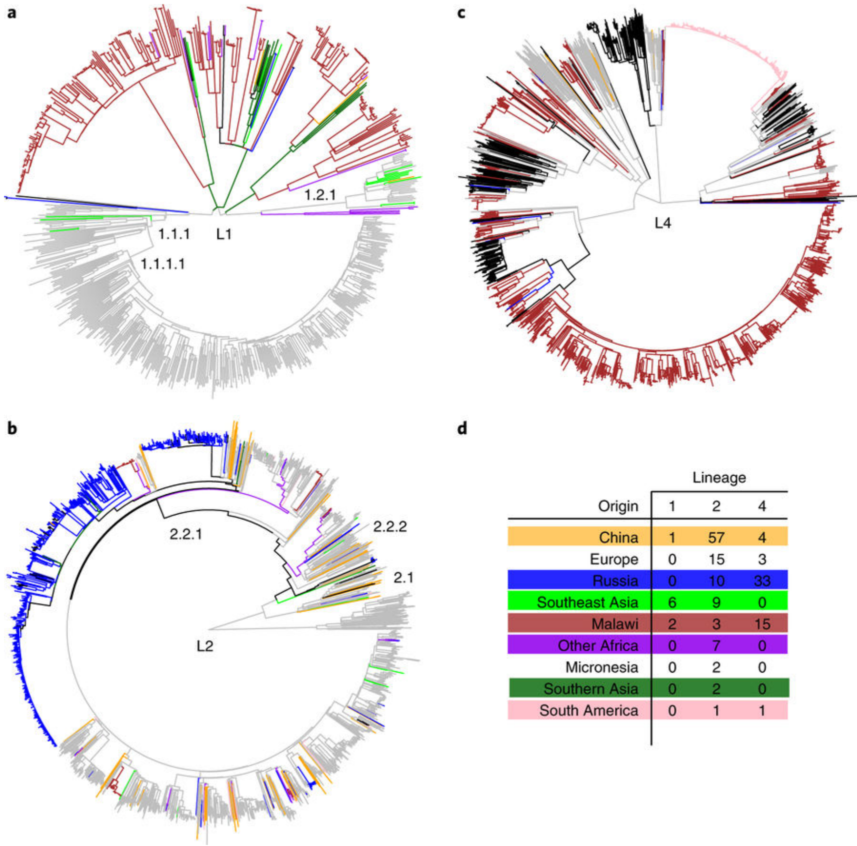
Local expansion of specific genotypes

ETHIOPIA



Comas et al 2015 Current Biology
Dots -> Ethiopia specific clades

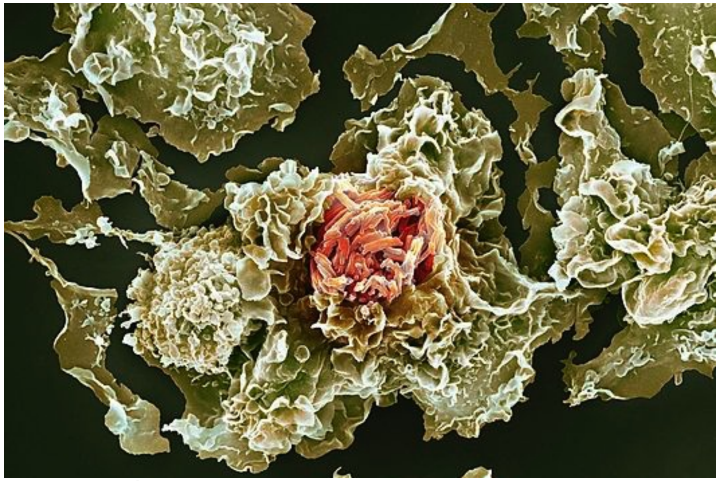
VIETNAM



	Lineage		
Origin	1	2	4
China	1	57	4
Europe	0	15	3
Russia	0	10	33
Southeast Asia	6	9	0
Malawi	2	3	15
Other Africa	0	7	0
Micronesia	0	2	0
Southern Asia	0	2	0
South America	0	1	1

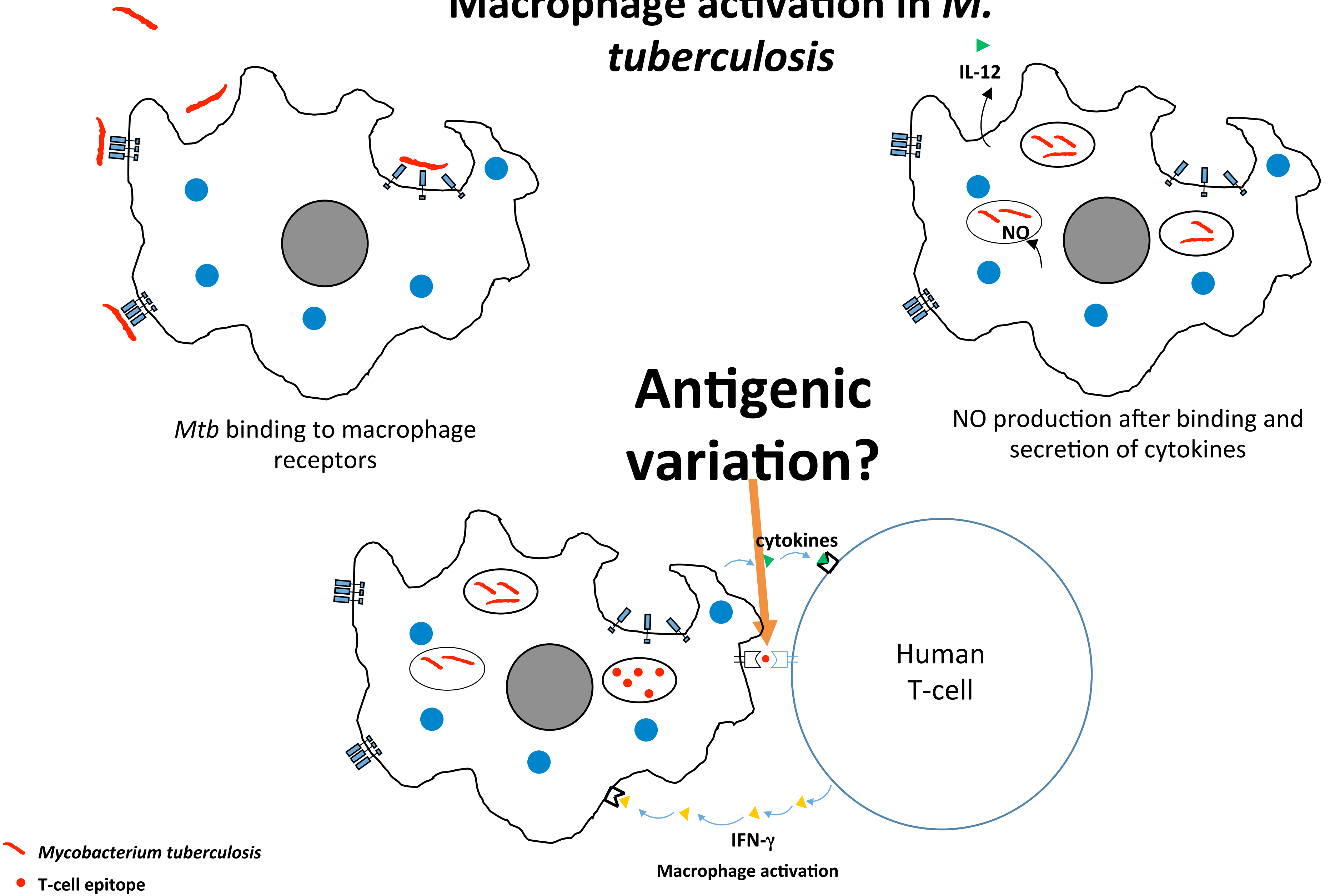
Holt et al Nature Genetics 2018
Grey -> Vietnam specific clades

Old and new selective pressure in MTBC evolution



Immune system

Macrophage activation in *M. tuberculosis*



Mtb binding to macrophage receptors

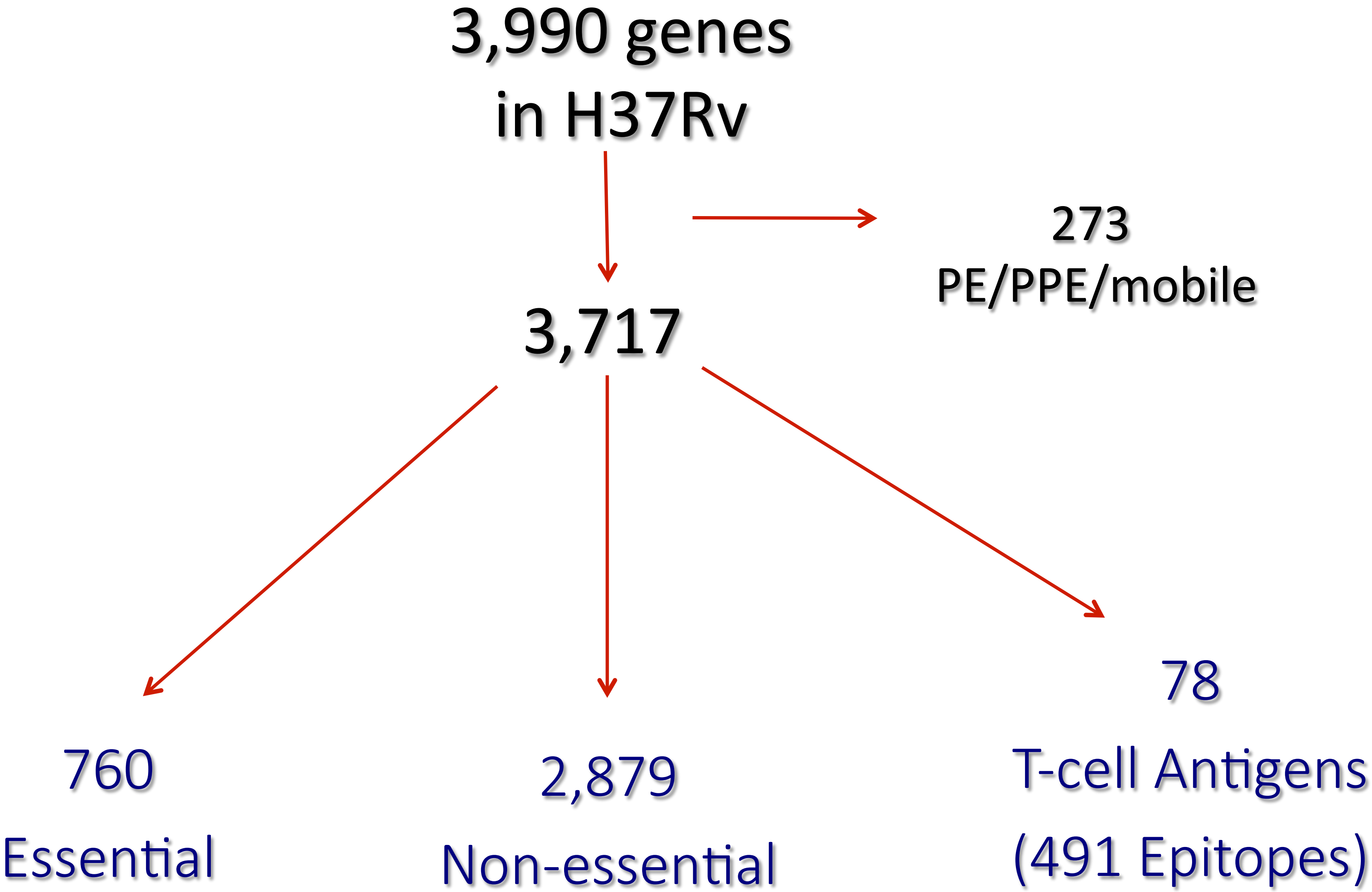
NO production after binding and secretion of cytokines

Antigenic variation?

Macrophage activation

- *Mycobacterium tuberculosis*
- ● **T-cell epitope**

Three (experimentally confirmed) Gene Sets:



3,990 genes
in H37Rv

273
PE/PPE/mobile

3,717

760
Essential

2,879
Non-essential

78
T-cell Antigens
(491 Epitopes)

dN/dS : rate of accumulation of non-synonymous versus accumulation of synonymous change

Nucleotide mutation

Synonymous : no amino acid change.
No effect on fitness.

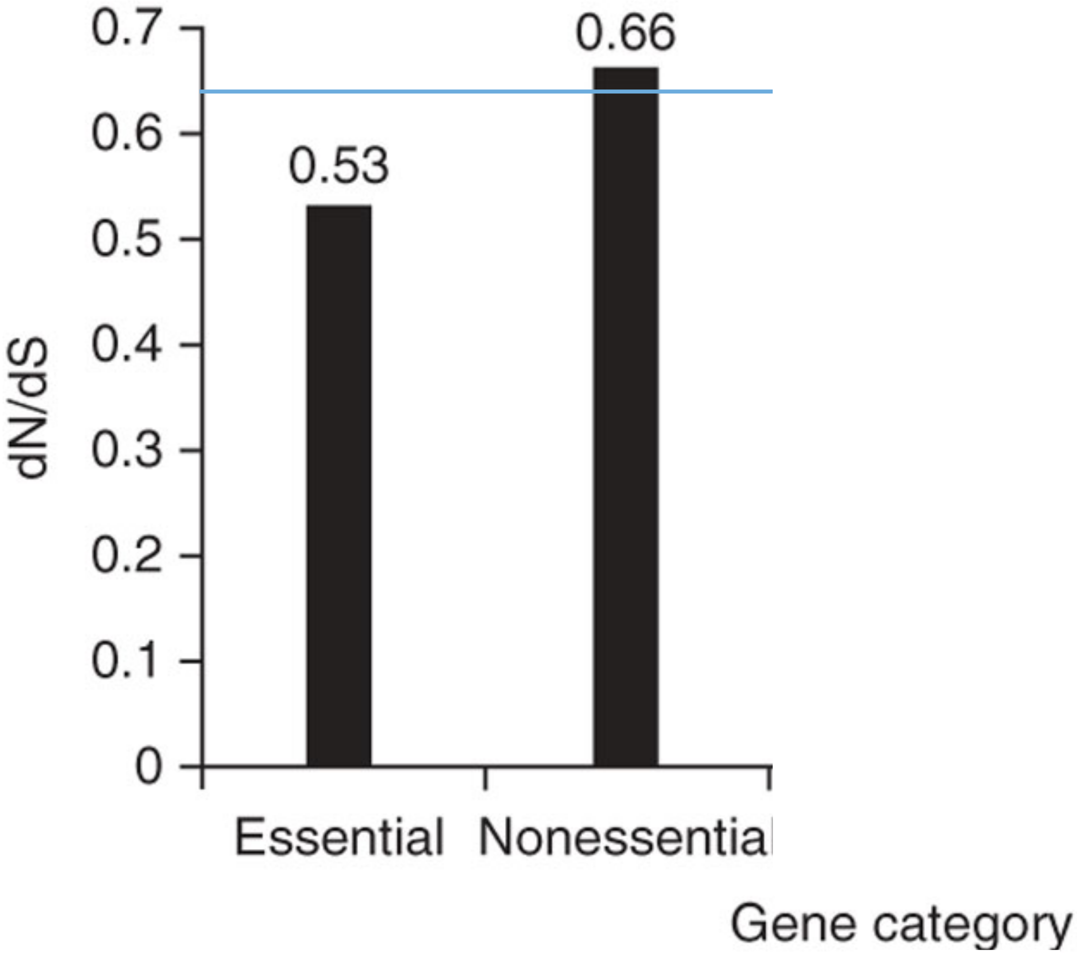
Non-synonymous : amino acid change.
Usually detrimental.

Low dN/dS (<<1) : PURIFYING SELECTION
(aminoacid conservation)

dN/dS (~1) : NO SELECTION just GENETIC DRIFT

dN/dS (>>1) : POSITIVE SELECTION

Epitopes Most Conserved



Comas et al. Nat. Genet. 2010

Conclusions

- Human T-cell epitopes of Mtb are hyperconserved (= essential genes).
- No immune evasion strategy: host immune responses benefit to *Mtb*?

Why *Mtb* needs a proper human T-cell immune response?

inhibits replication

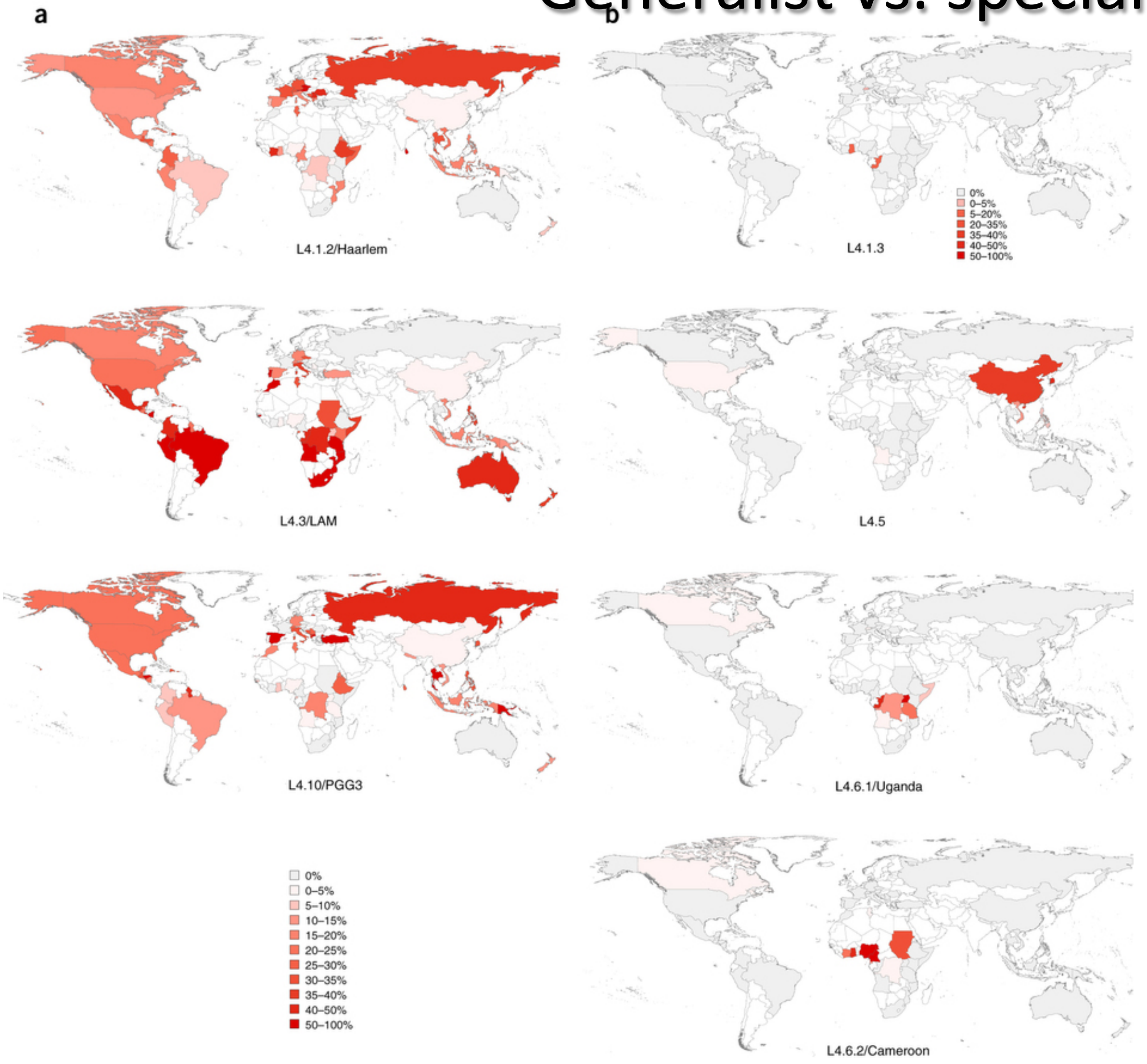
lung damage

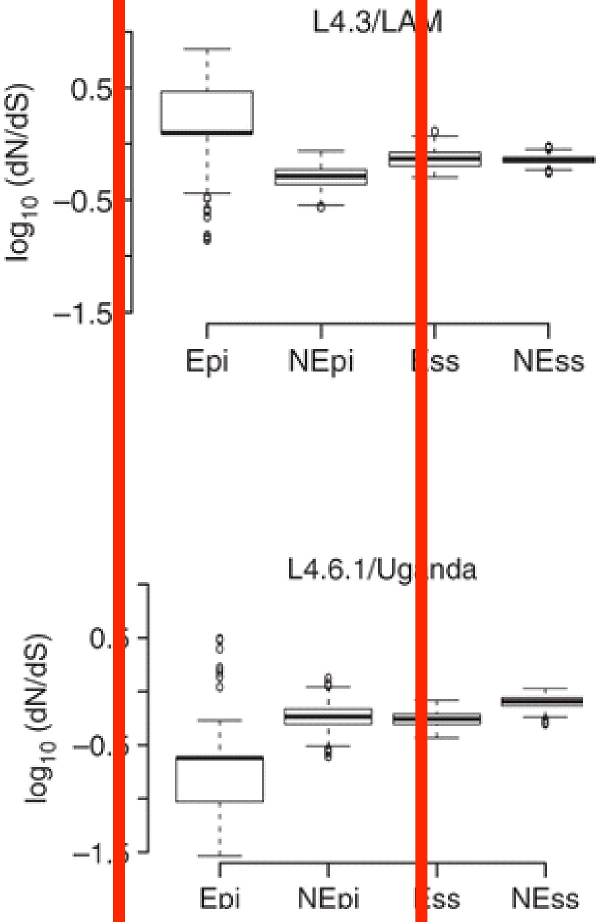
transmission

Conclusions

- (Known) Human T-cell epitopes of Mtb are hyperconserved (= essential genes).
- No immune evasion strategy: some host immune responses benefit to *Mtb*?
- Implications for vaccines: what constitutes protective immunity?
- New antigens? -> yes and no -> Coscolla Cell Host and Microbe 2015

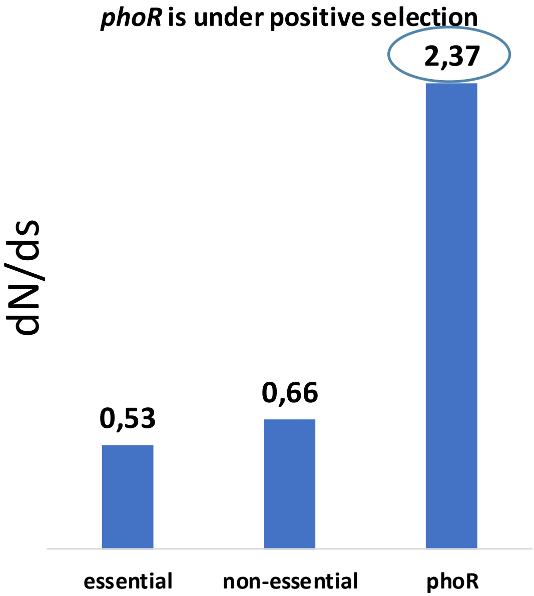
Generalist vs. specialist?





Stucki, Brites et al. Nat Genet 2016

Positive selection not in antigens, yes in virulence factors


phoR is under positive selection

PhoR

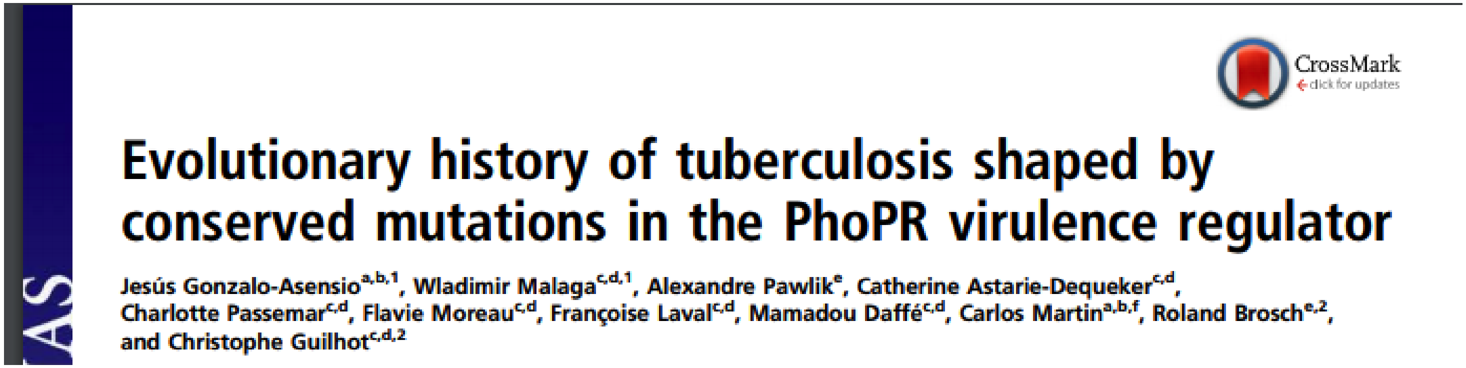

Review

Cell PRESS

PhoP, a key player in *Mycobacterium tuberculosis* virulence

Michelle Ryndak[1], Shuishu Wang[2] and Issar Smith[1,3]

MTBVAC: live-attenuated vaccine based on PhoP deletion under clinical trials


CrossMark click for updates

Evolutionary history of tuberculosis shaped by conserved mutations in the PhoPR virulence regulator

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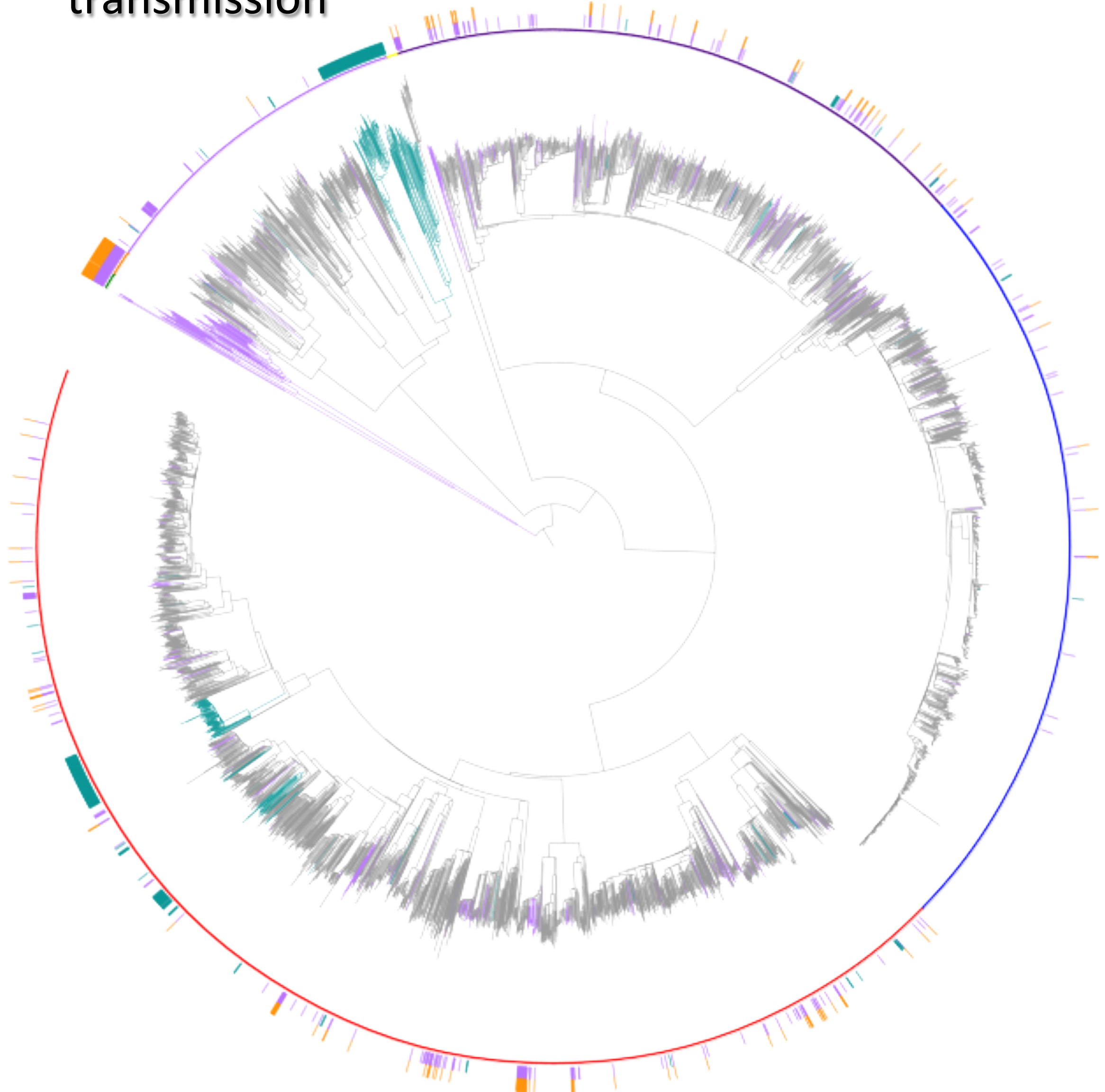
Early mutations in PhoR associated to host-range adaptation

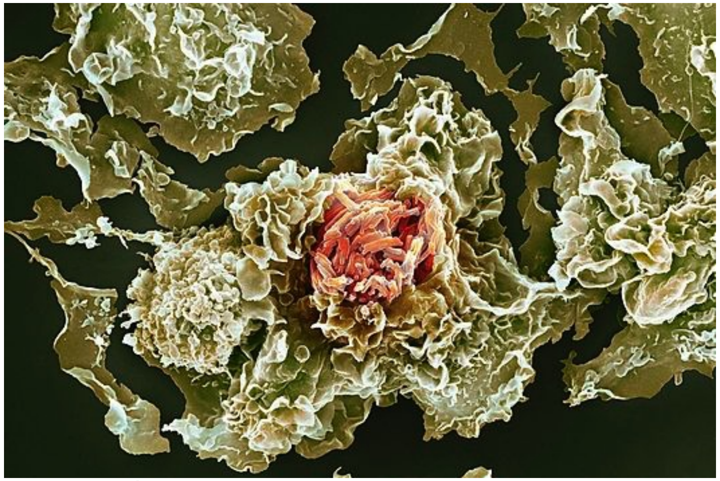
phoR mutations are prevalent across the MTBC phylogeny and associated to on-going transmission



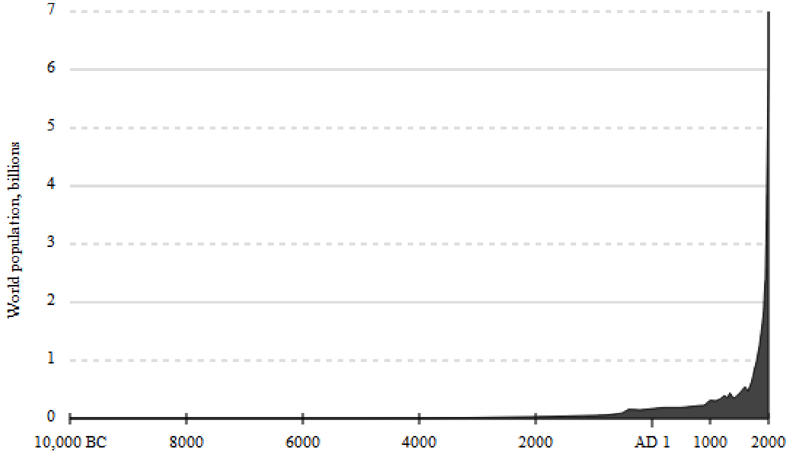
Associated to large transmission clusters in Malawi
(pvalue < 0,05)

Chiner-Oms et al 2018 Bioarxiv

Old and new selective pressure in MTBC evolution



Immune system

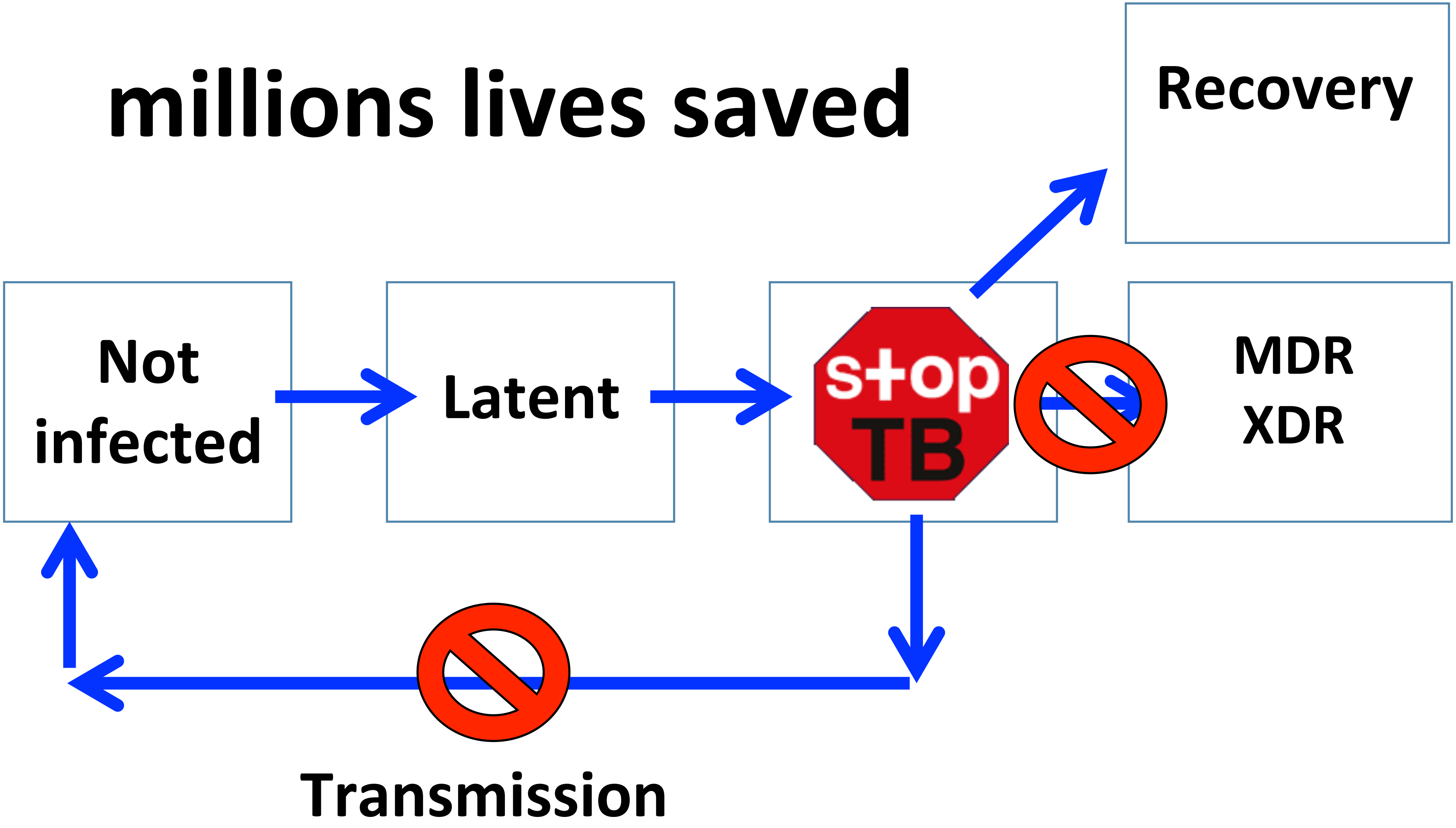


Human population

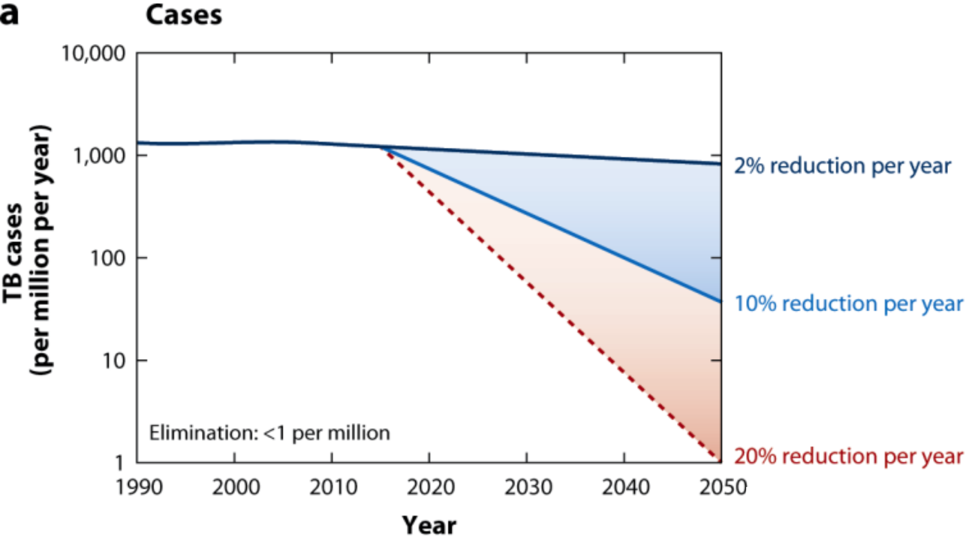
Modern epidemics

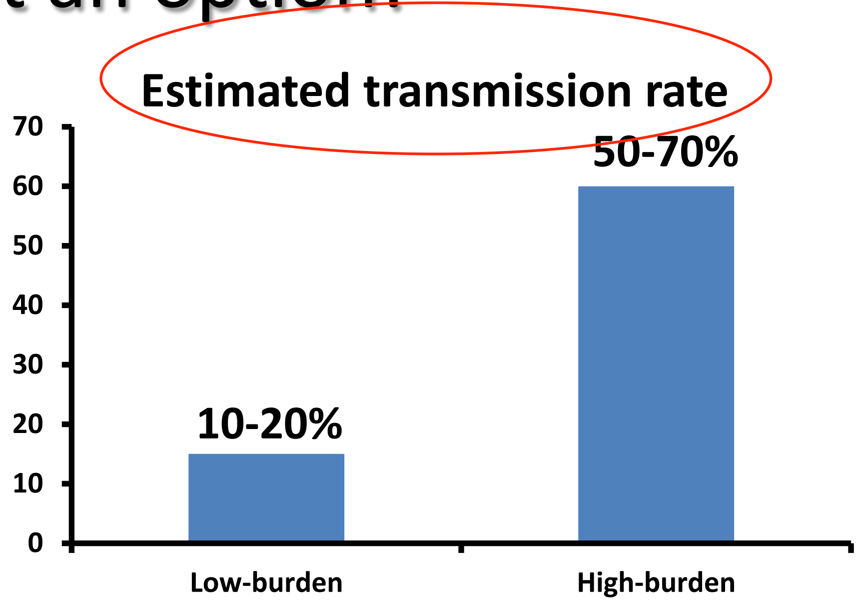
DOTS: Directly Observed Treatment, Short-Course

millions lives saved



Slow and Steady is not an option!





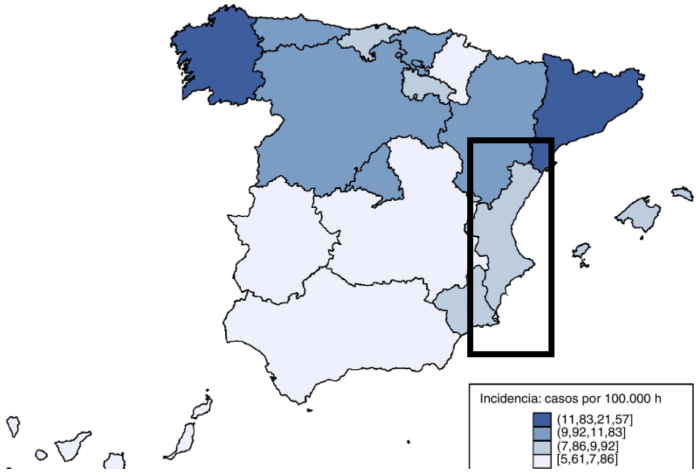
Dye 2013

— Current rate
▨ Possible with current technology
▨ Beyond current technology

- **Decades old tools**
- **Inaccurate epidemiological models**
- **Knowledge gaps on host-pathogen**

- **Transmission is on-going!**
- **Contributes to latent reservoir -> future epidemics**

Low-burden setting: Valencia region



Incidence: 8/100,000 inhabitants

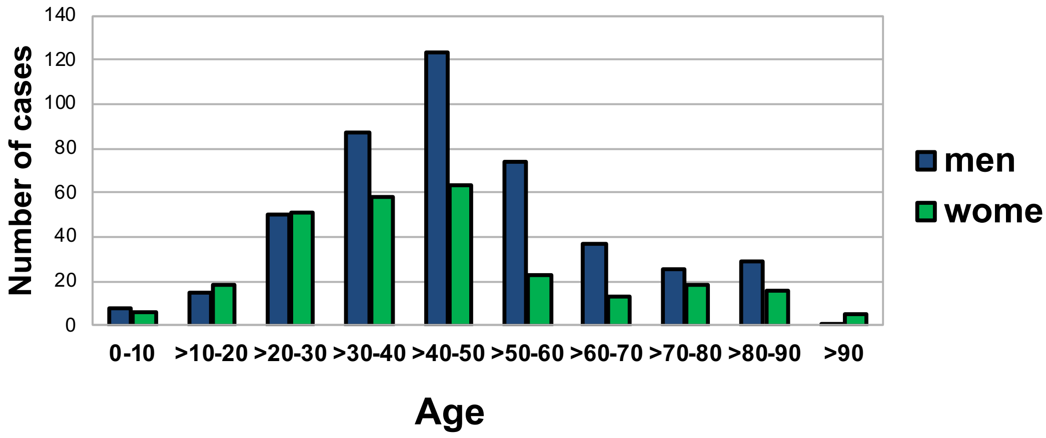
Period: 2014-2016

785 sequenced isolates

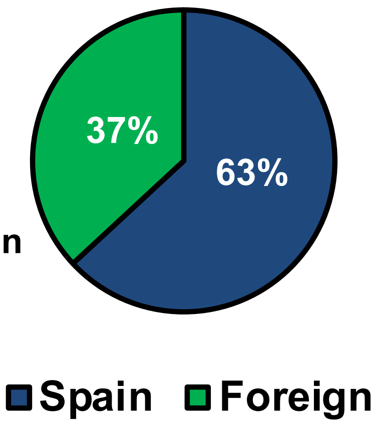
18 Hospitals

3 locations:

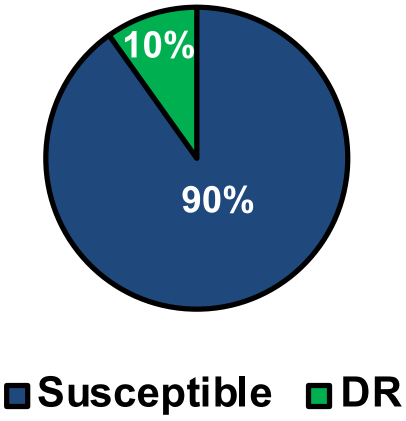
- **Castellón**
- **Valencia**
- **Alicante**


TB cases per age and gender

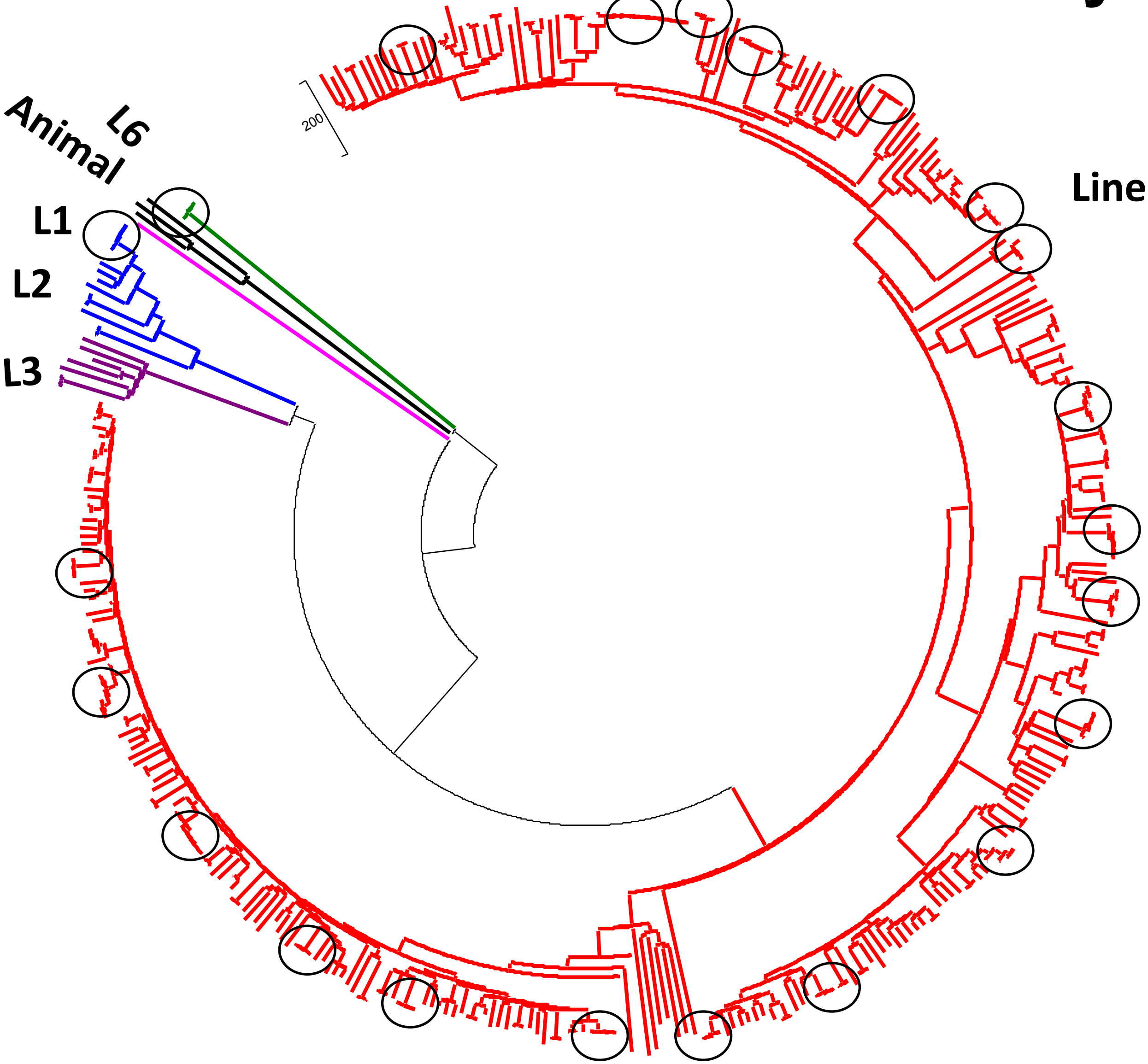
Country of origin



Resistance



Valencia MTBC Phylogeny



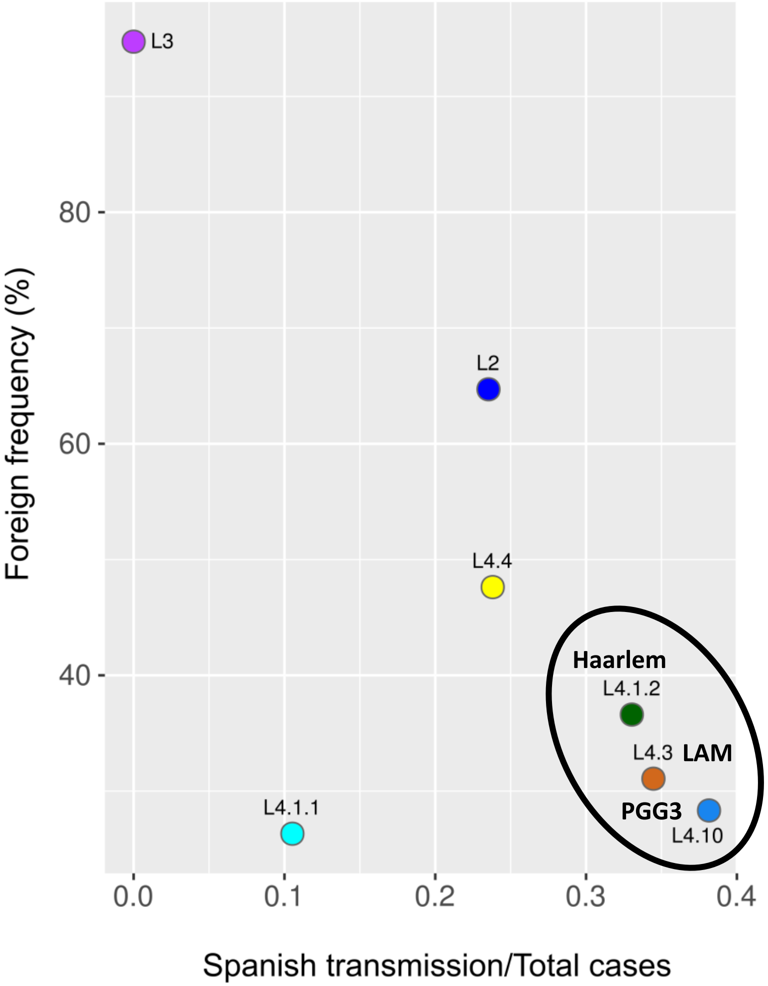
Lineage 4 **785 isolates**

Lineage*	Cases	No. Clusters
L1	4	0
L2	21	2
L3	20	0
L4	**717**	**118**
L5	2	0
L6	3	1
Animal	13	0

Cancino et al. Unpublished

* Nomenclature proposed by Comas, et al. *Nat Genet.* 2013

Generalist sub-lineages associated to local transmission



But sub-lineages are a large pool of imported and indigenous strains. Can we distinguish?

1. Identify genotypes beyond sub-lineage level
2. Correlate the genotypes with Spanish origin

Cancino et al. Unpublished

* Nomenclature proposed by Stucki, et al. *Nat Genet.* 2016

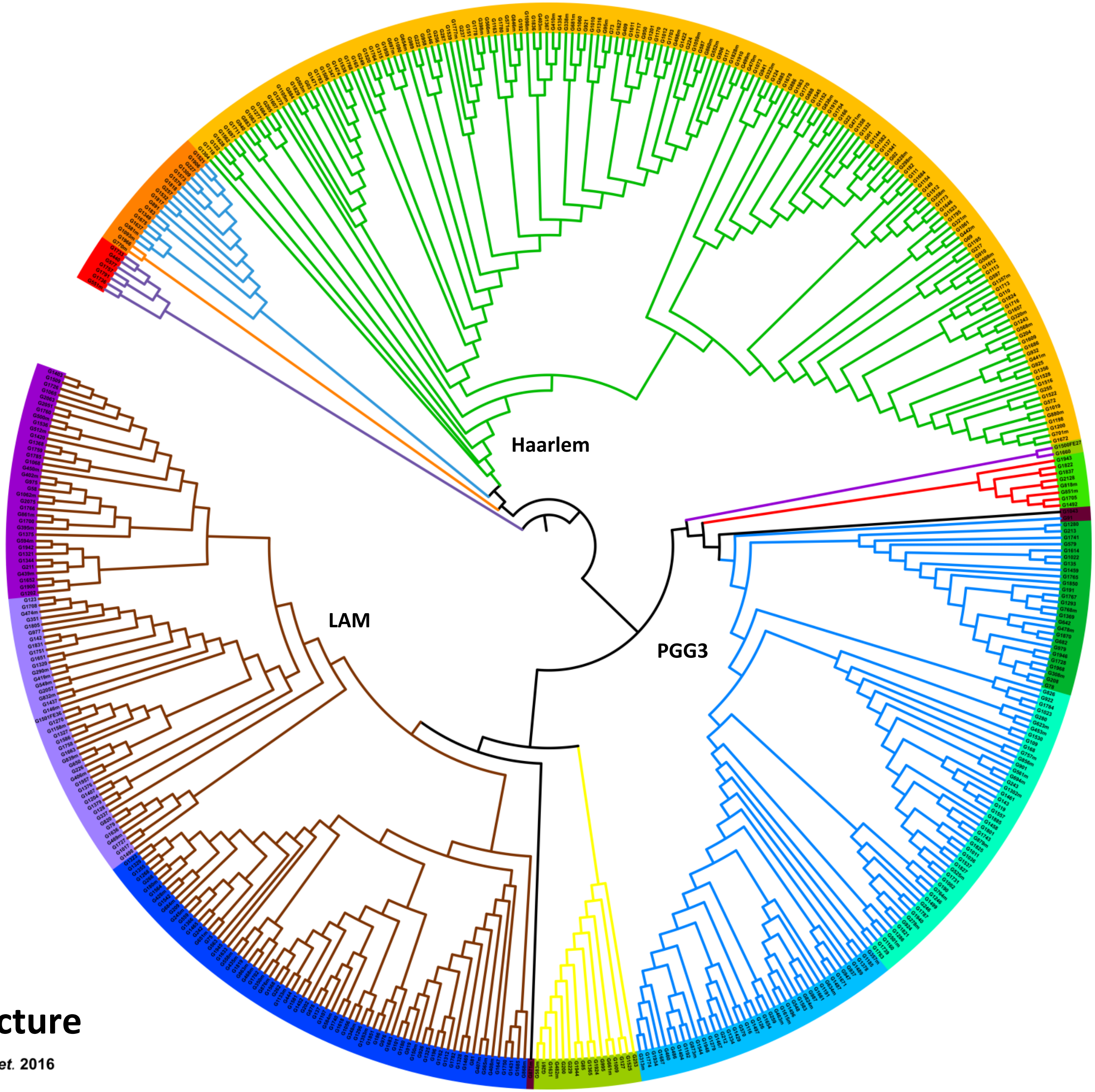
1. Valencia local population structure: beyond sub-lineages

Valencia Cluster

- 🟥 Val CL01
- 🟧 Val CL02
- 🟨 Val CL03
- 🟩 Val CL04
- 🟩 Val CL05
- 🟩 Val CL06
- 🟦 Val CL07
- 🟦 Val CL08
- 🟦 Val CL09
- 🟪 Val CL10
- 🟪 Val CL11
- 🟫 Val CL12

Sub-lineages*

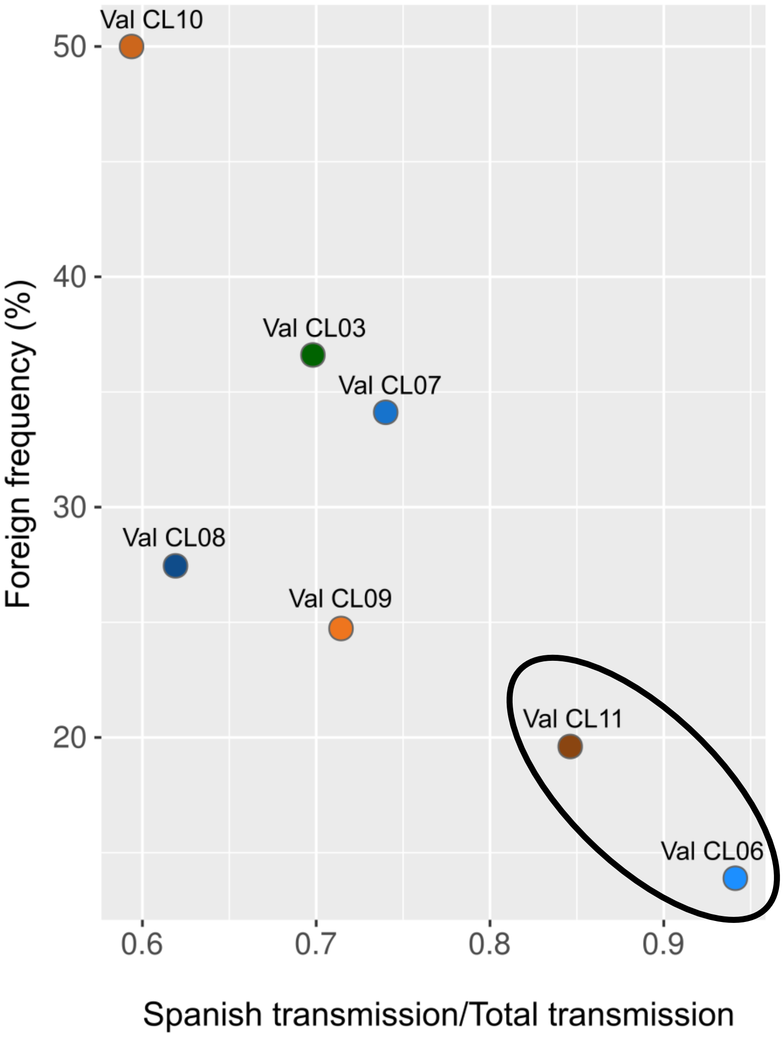
- L4.2
- L4.1.3
- L4.1.1
- L4.1.2
- L4.5
- L4.6.2
- L4.10
- L4.4
- L4.3



hBAPS to define local structure

*Nomenclature proposed by Stucki et al. *Nat Genet.* 2016

2. Indigenous genotypes associated to transmission



Cluster	Total transmission	Spanish/Total transmission (%)	Odds ratio	95% CI	*p* value*
Val CL03	106	69.81	1.4	(0.65-3.6)	0.247
Val CL06	**17**	**94.12**	**10.5**	**(1.6-492.4)**	**0.009**
Val CL07	50	74.00	1.9	(0.78-5.5)	0.126
Val CL08	21	61.9	1.1	(0.37-4.0)	0.542
Val CL09	42	71.43	1.6	(0.67-5.0)	0.200
Val CL10	32	59.37		Reference	
Val CL11	**26**	**84.61**	**3.6**	**(1.1-18.1)**	**0.033**

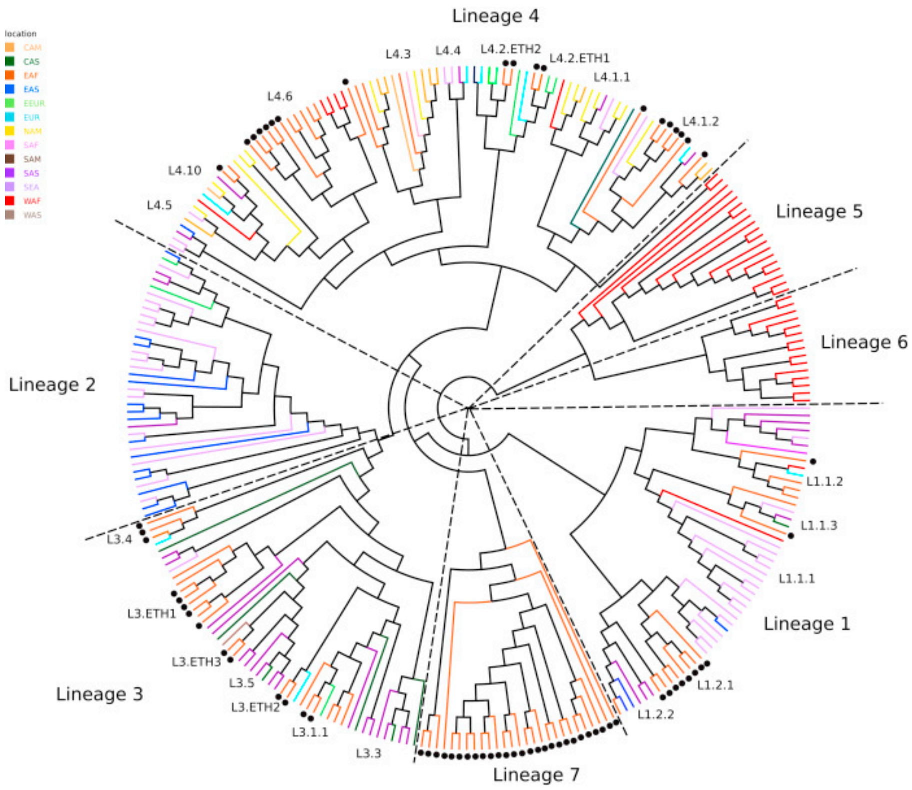
*Fisher exact test

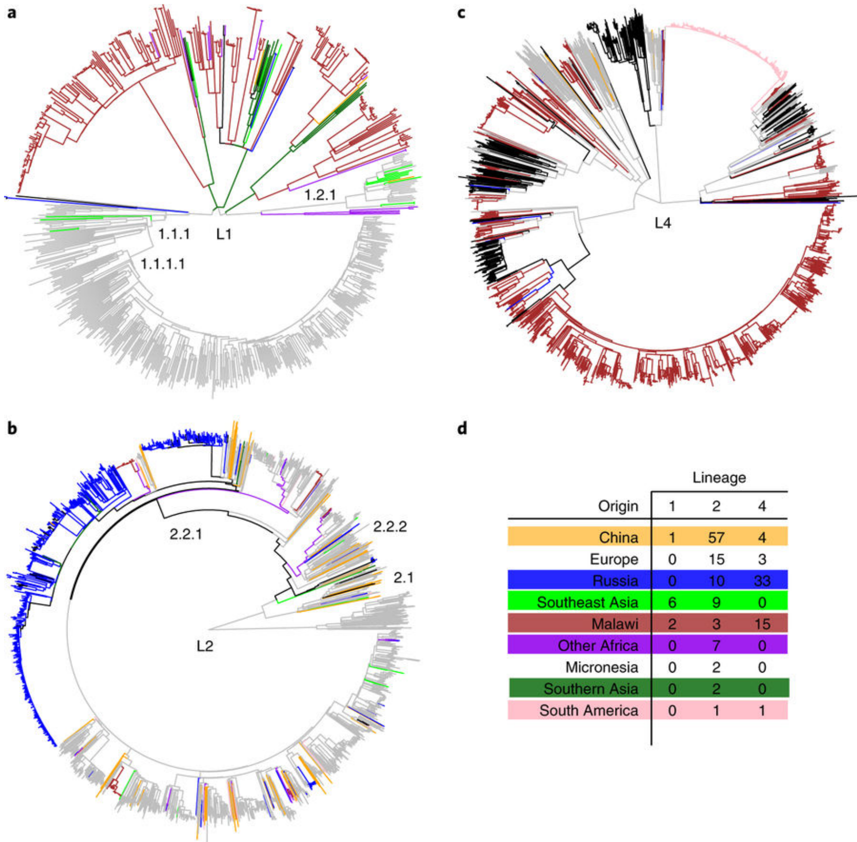
Cancino et al. Unpublished

Local expansion of specific genotypes driving transmission

ETHIOPIA

VIETNAM





	Lineage		
Origin	1	2	4
China	1	57	4
Europe	0	15	3
Russia	0	10	33
Southeast Asia	6	9	0
Malawi	2	3	15
Other Africa	0	7	0
Micronesia	0	2	0
Southern Asia	0	2	0
South America	0	1	1

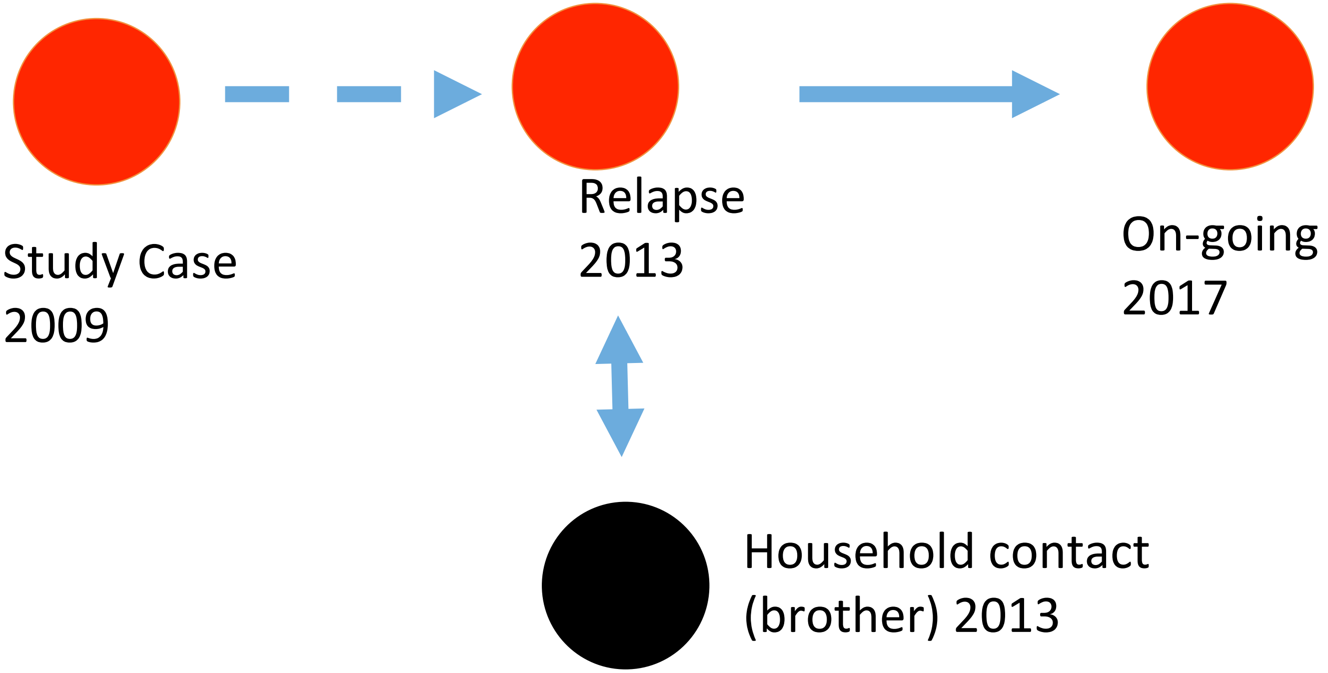
Comas et al 2015 Current Biology
Dots -> Ethiopia specific clades

Holt et al Nature Genetics 2018
Grey -> Vietnam specific clades

Old and new selective pressure in MTBC evolution

Antibiotics

Whole genome sequencing for drug resistance detection



Study Case
2009

Relapse
2013

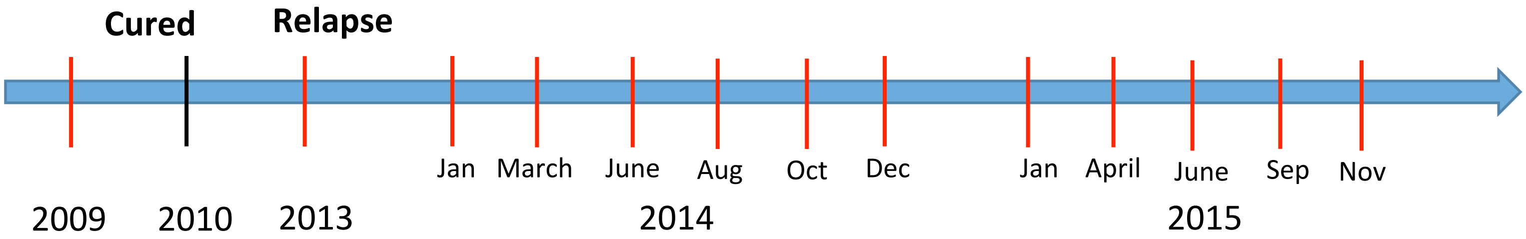
On-going
2017

Household contact
(brother) 2013

DISCLAIMER: all sequencing data has been obtained from culture isolates ☹

Cancino et al. Under Review

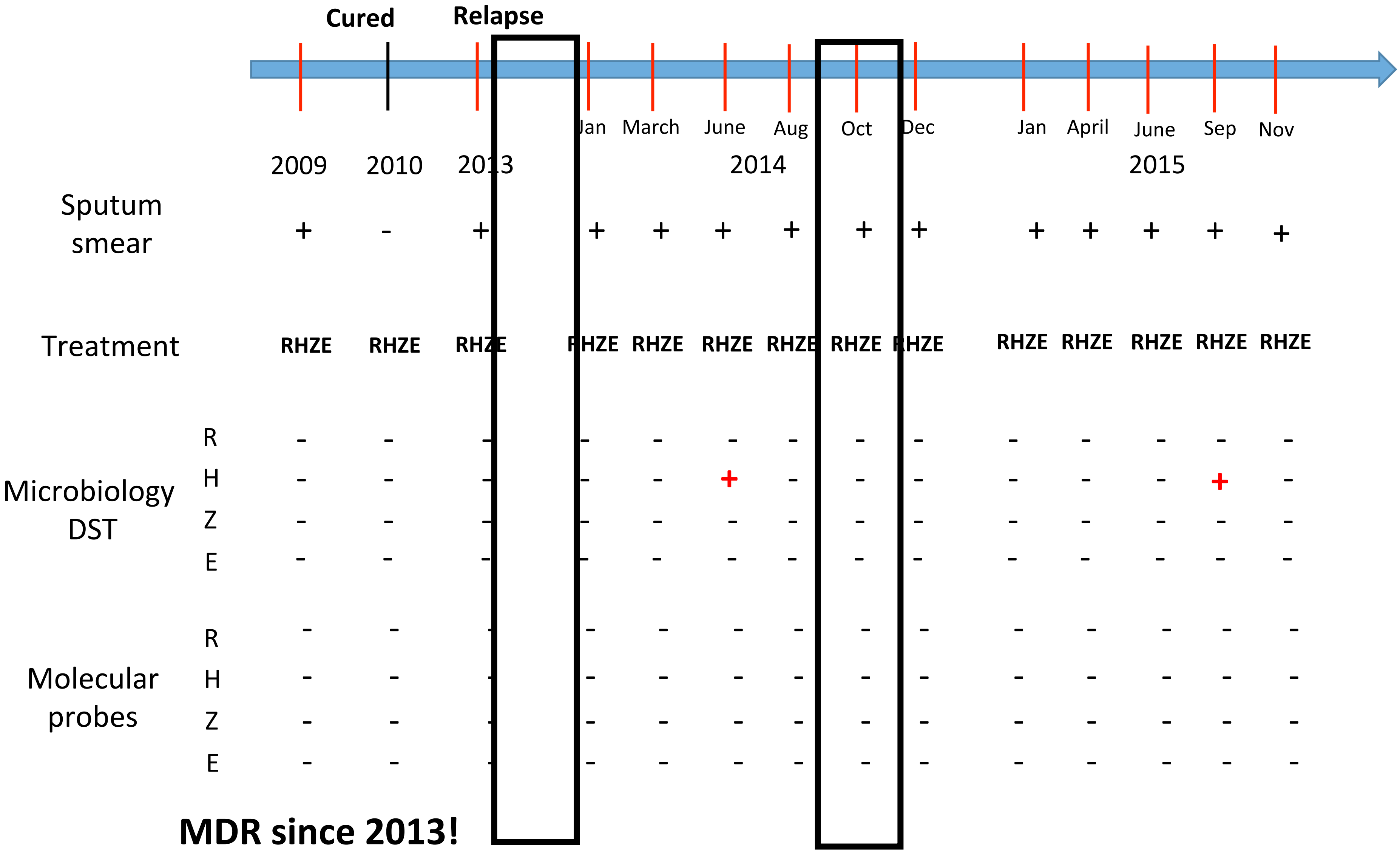
Microbiological and molecular diagnostic



		2009	2010	2013	2014 Jan	2014 March	2014 June	2014 Aug	2014 Oct	2014 Dec	2015 Jan	2015 April	2015 June	2015 Sep	2015 Nov
Sputum smear		+	-	+	+	+	+	+	+	+	+	+	+	+	+
Treatment		**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**	**RHZE**
Microbiology DST	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	H	-	-	-	-	-	+	-	-	-	-	-	-	+	-
	Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Molecular probes	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-

R, Rifampicin; H, Isoniazid; Z, Pyrazinamide; E, Ethambutol

Microbiological and molecular diagnostic



		2009	2010	2013	2014 Jan	2014 March	2014 June	2014 Aug	2014 Oct	2014 Dec	2015 Jan	2015 April	2015 June	2015 Sep	2015 Nov
Sputum smear		+	-	+	+	+	+	+	+	+	+	+	+	+	+
Treatment		RHZE	RHZE	RHZE	RHZE	RHZE	RHZE	RHZE	RHZE	RHZE	RHZE	RHZE	RHZE	RHZE	RHZE
Microbiology DST	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	H	-	-	-	-	-	+	-	-	-	-	-	-	+	-
	Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Molecular probes	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Z	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MDR since 2013!

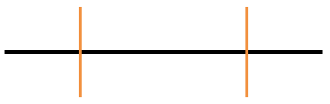
MDR diagnosed by WGS

R, Rifampicin; H, Isoniazid; Z, Pyrazinamide; E, Ethambutol

Why diagnostics failed?

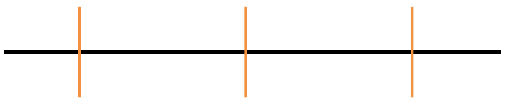
Molecular

rpoB (RIF)



I491F

katG (INH)



G273R 1 bp del

GenExpert Hain Others	**None**

GenExpert Hain Others	**None**

MGIT

False negative
(slow growth due to I491F "disputed mutation")

RIFAMPICIN

MGIT	< 1 ug/ul
7H11	> 1 ug/ul

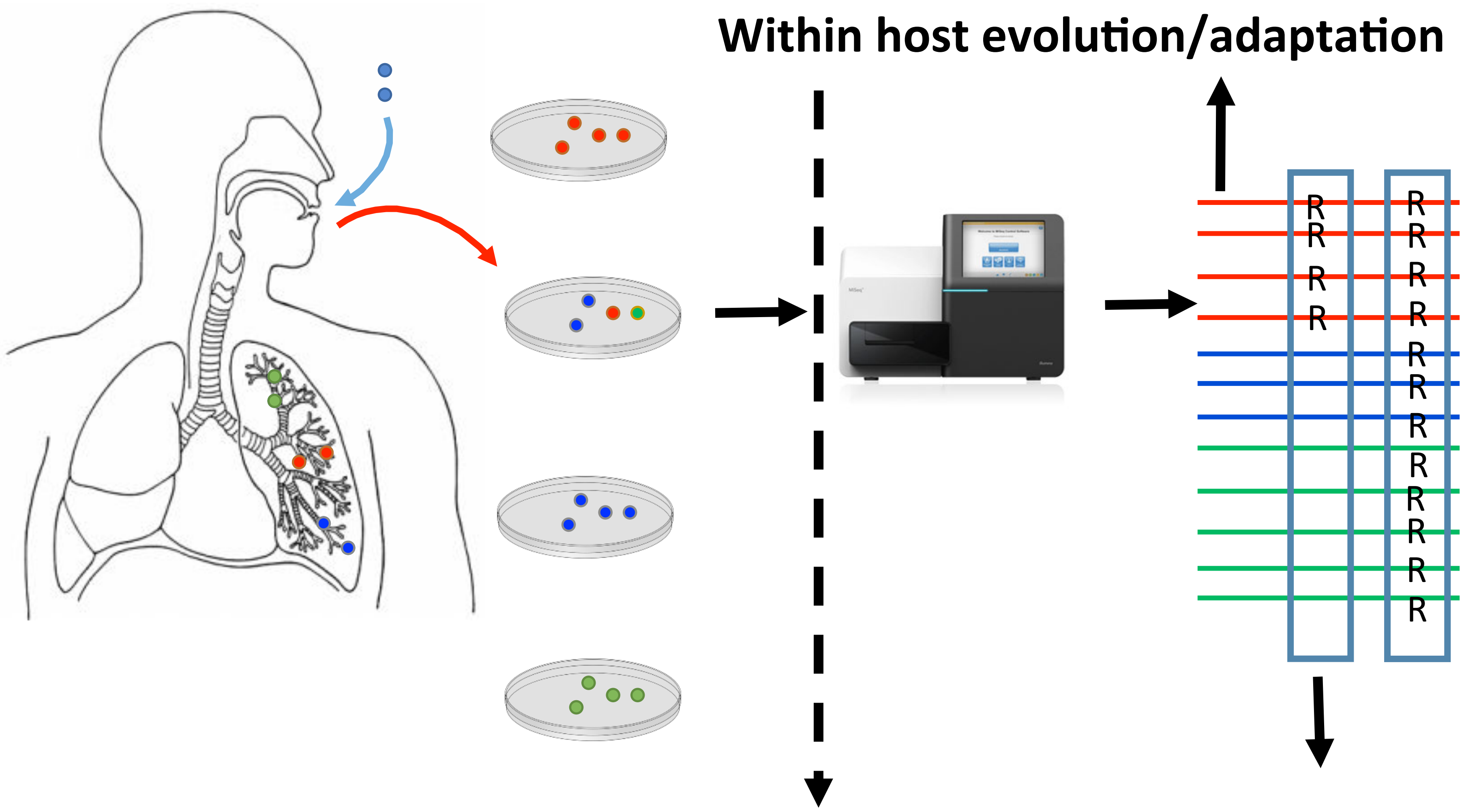
Breakpoint

ISONIAZID (MGIT -)

Strain	S/R?	0	0,125	0,25	0,5	1	8	16	32
H37Rv	control_S	🟥	🟥	🟥	🟦	🟦	🟦	🟦	🟦
G1480	2009_S	🟥	🟥	🟥	🟦	🟦	🟦	🟦	🟦
G520_C2	2014_R	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥

Cancino et al. Under Review

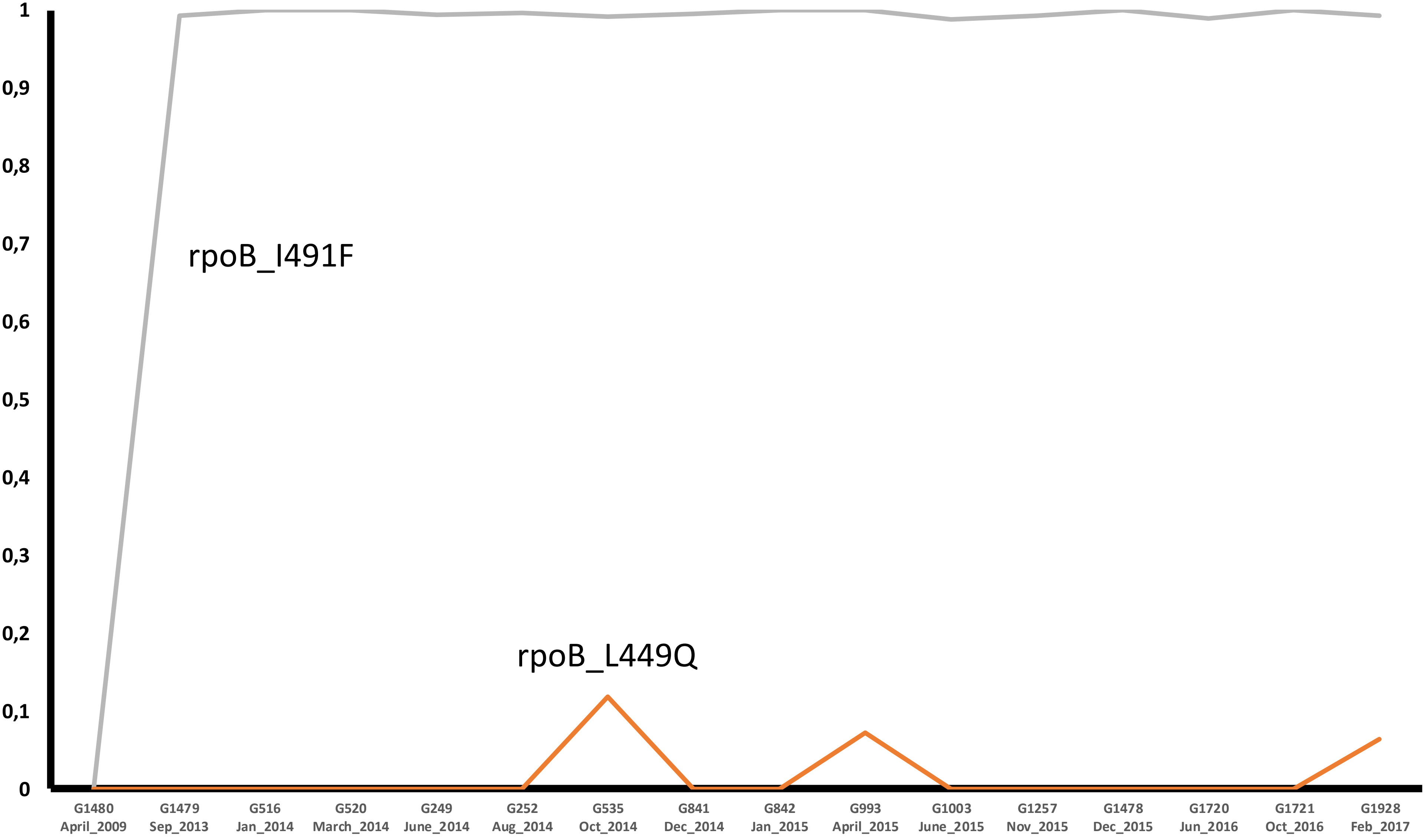
Complexity of infections AND heteroresistance



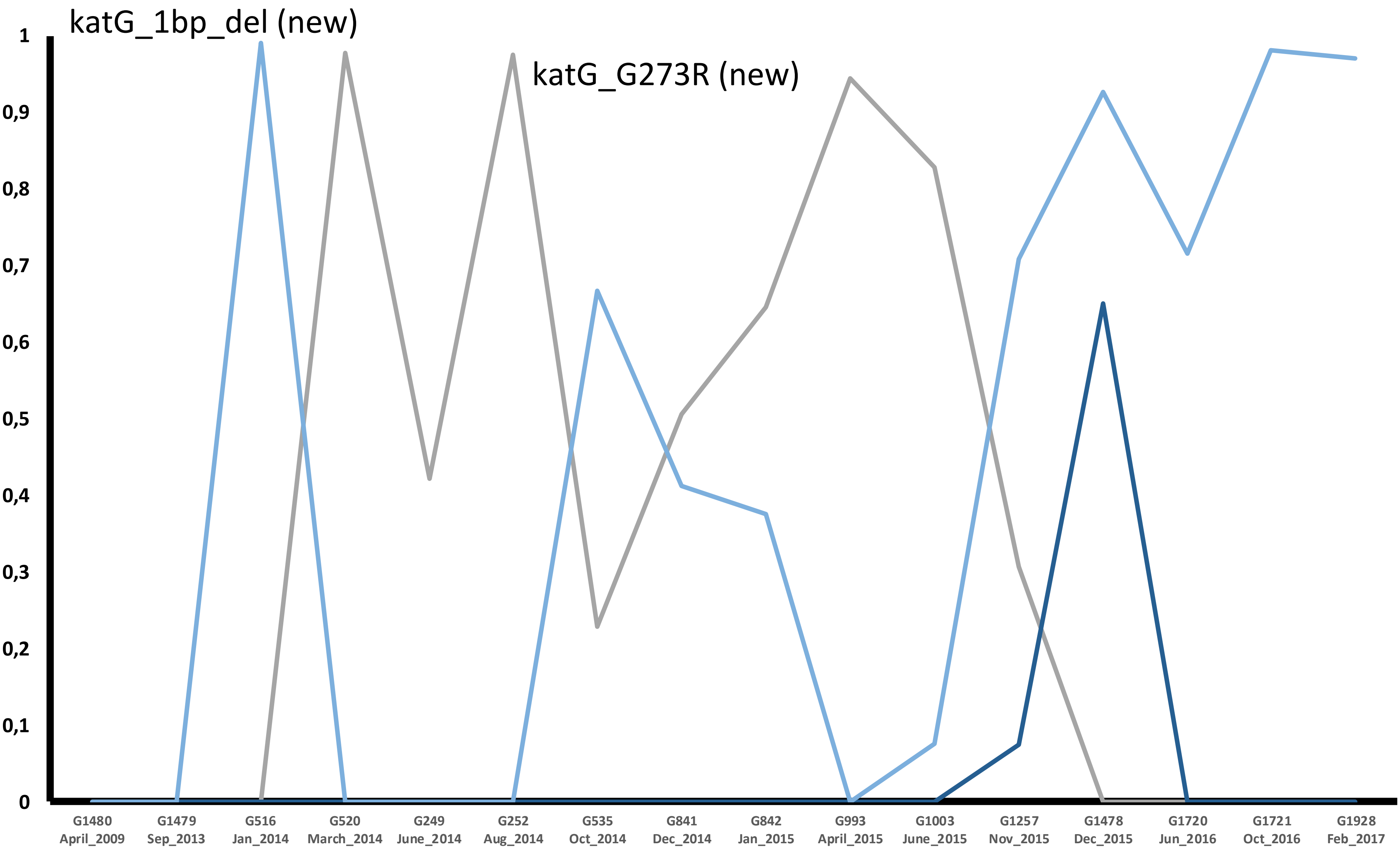
Within host evolution/adaptation

Clinically relevant?

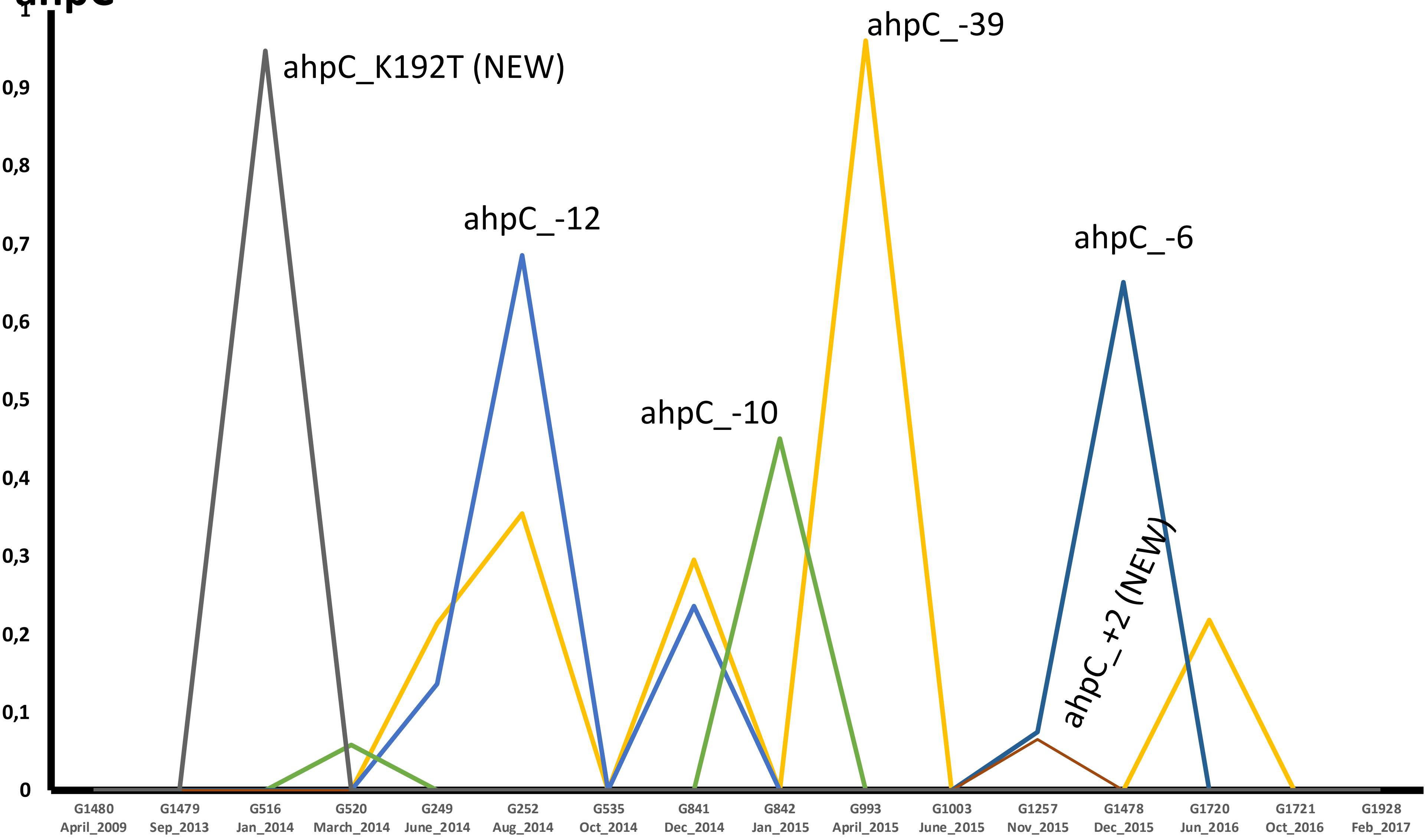
Resistance to Rifampicin – Sep 2013



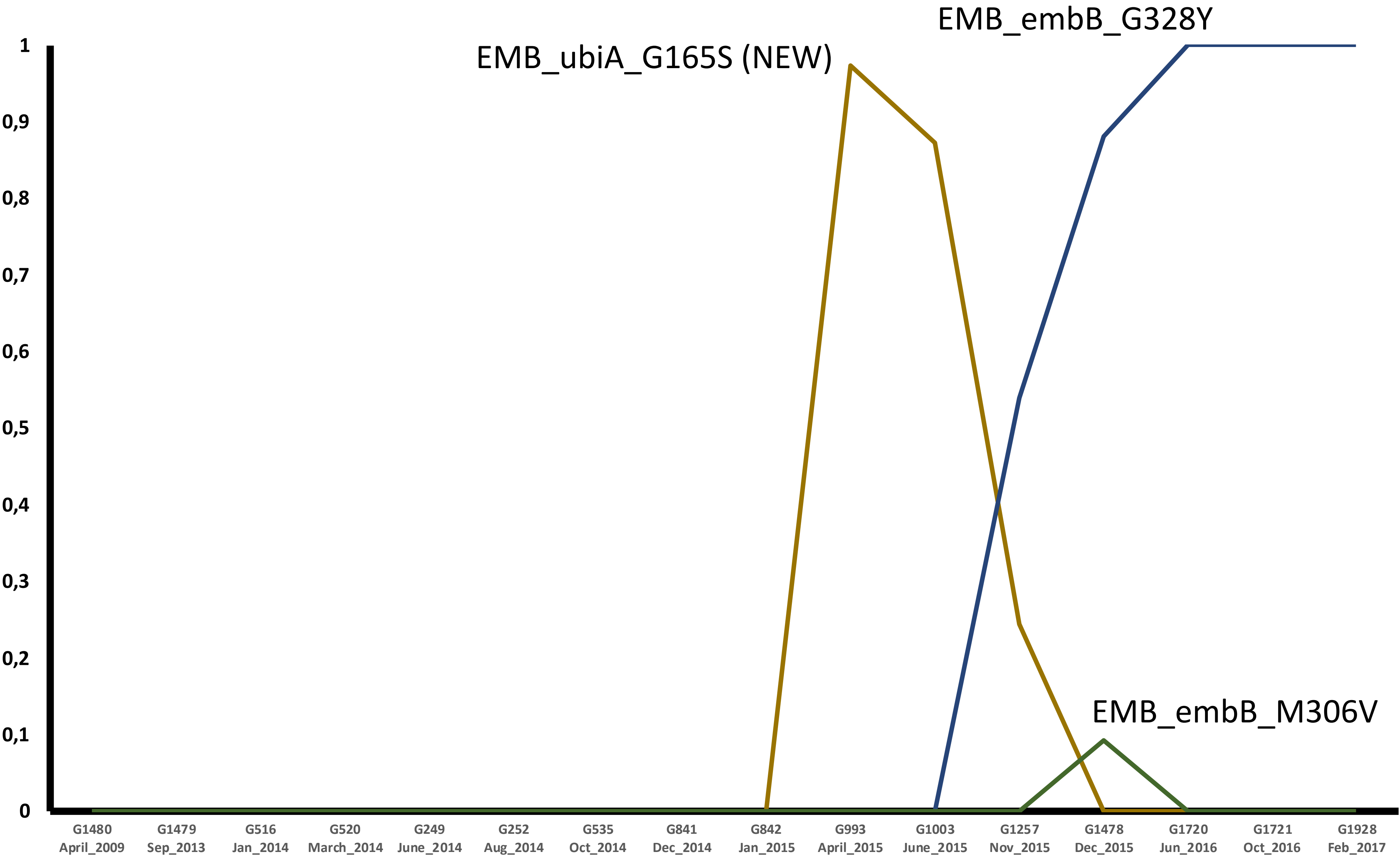
Resistance to
Rifampicin – Sep 2013
Isoniazid – Jan 2014



Resistance to Rifampicin – Sep 2013 Isoniazid – Jan 2014 + mutations in ahpC

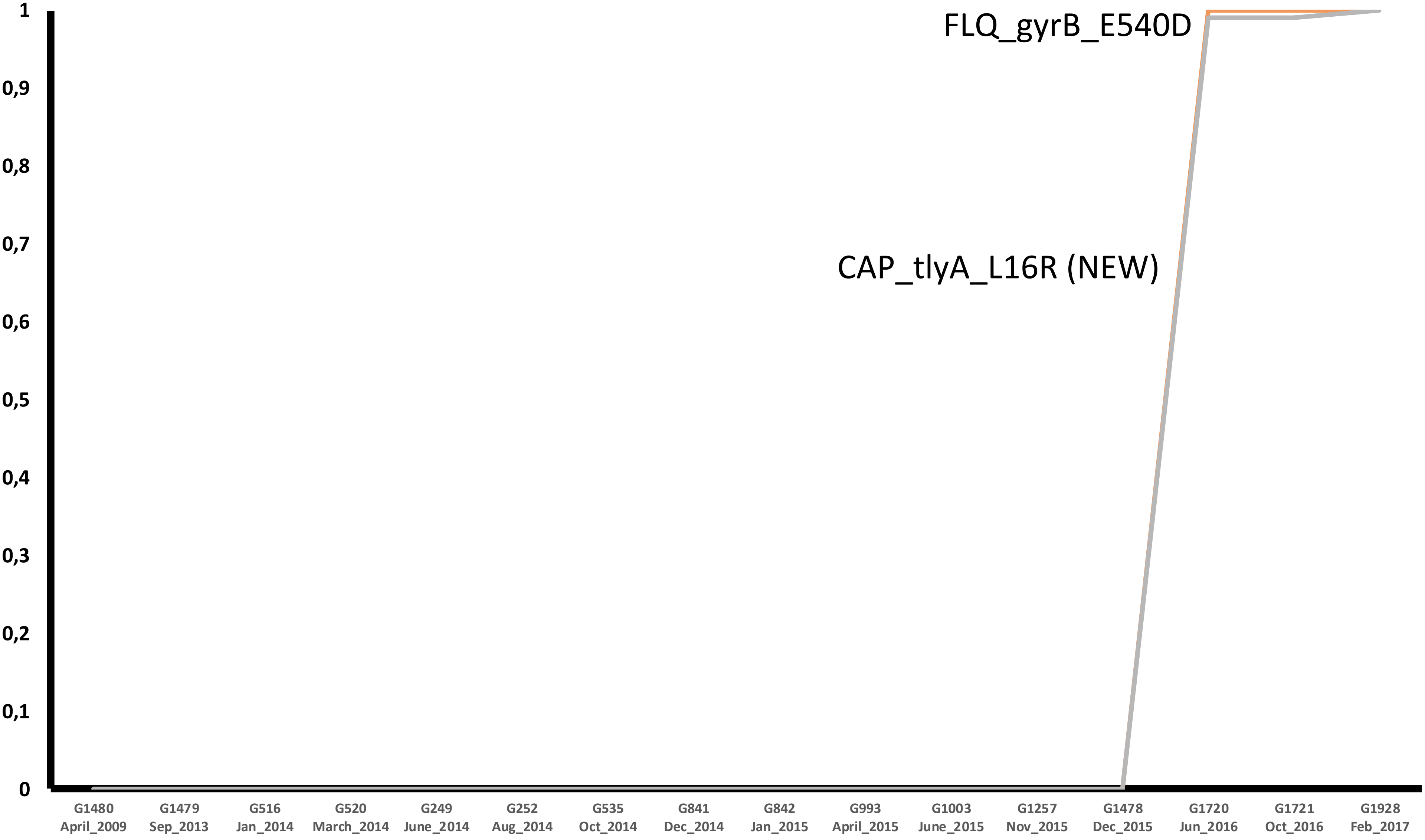


Resistance to
Rifampicin – Sep 2013
Isoniazid – Jan 2014
Ethambutol – Jan 2015



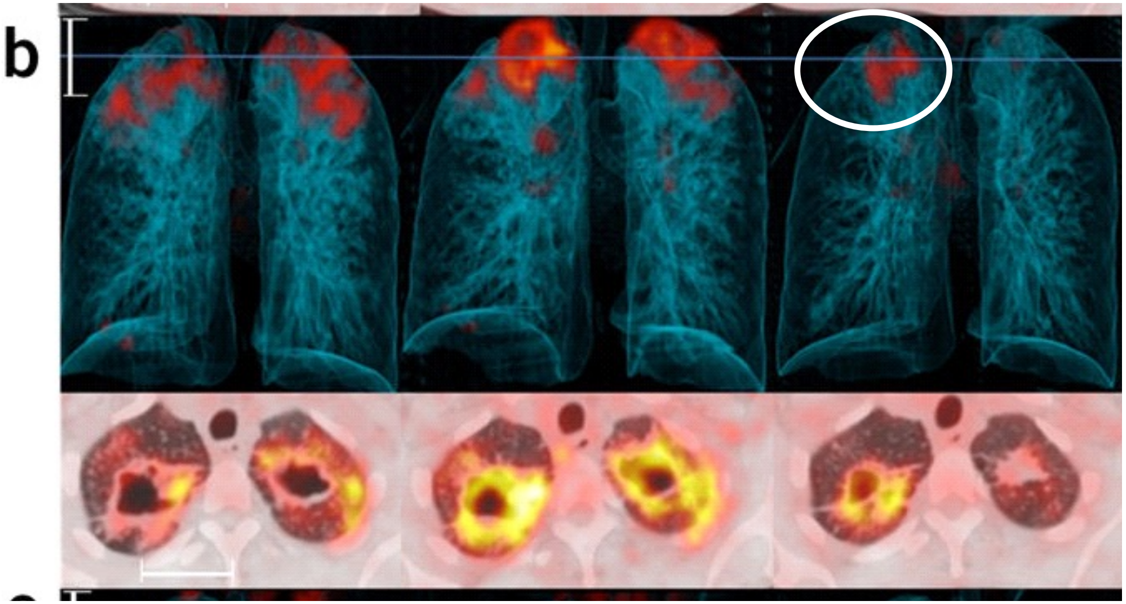
Resistance to
Rifampicin – Sep 2013
Isoniazid – Jan 2014

Ethambutol – Jan 2015
FQ + CAPREO – Jun 2016

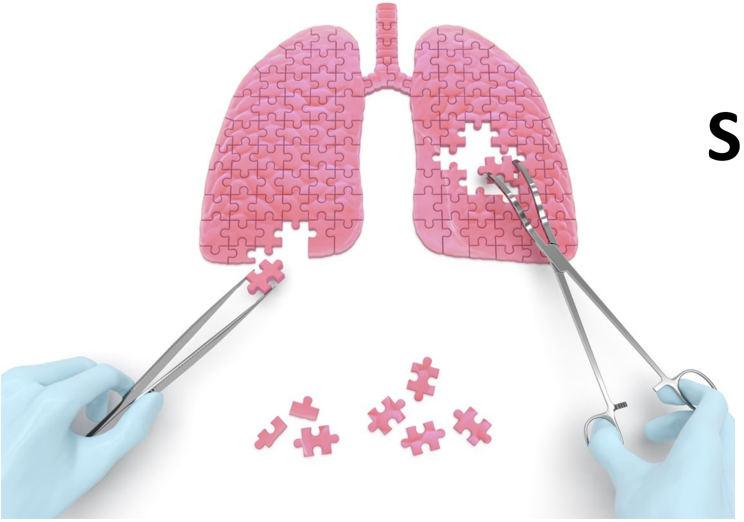



G1480 April2009
G1479 Sep2013
G516 Jan2014
G520 March2014
G249 June2014
G252 Aug2014
G535 Oct2014
G841 Dec2014
G842 Jan2015
G993 April2015
G1003 June2015
G1257 Nov2015
G1478 Dec2015
G1720 Jun2016
G1721 Oct2016
G1928 Jan2017
Treatment
Rifampicin
Isoniazid
Ethambutol
Pyrazinamide
Moxifloxacin
Capreomycin
Linezolid
WGS resistance profile
Susceptible
Rifampicin
Isoniazid
Ethambutol
Moxifloxacin
Capreomycin
Resistance type
Susceptible
MDR
MDR + EMBr
pre XDR + EMBr
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
G1480 April_2009
G1479 Sep_2013
G516 Jan_2014
G520 March_2014
G249 June_2014
G252 Aug_2014
G535 Oct_2014
G841 Dec_2014
G842 Jan_2015
G993 April_2015
G1003 June_2015
G1257 Nov_2015
G1478 Dec_2015
G1720 Jun_2016
G1721 Oct_2016
G1928 Feb_2017
RIF_rpoB_I491F
RIF_ropB_L449Q
INH_katG_G273R (NEW)
INH_AhpC_OxyR_(-39)
INH_AhpC_OxyR_(-12)
INH_AhpC_OxyR_(-10)
INH_AhpC_OxyR_(-6)
INH_AhpC_OxyR_(+2) (NEW)
INH_AhpC_K192T (NEW)
EMB_ubiA_G165S (NEW)
EMB_embB_G328Y
EMB_embB_M306V
INH_katG_1pb_DEL(NEW)
FLQ_gyrB_E540D
CAPRE_tlyA_L16R (NEW)

But, What is in your lung?

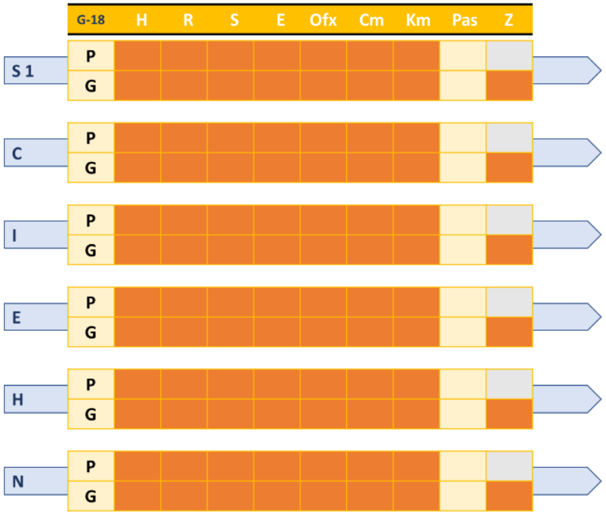


Example of on-going lesions after regular 6 months
Malherbe et al. Nat Med 2016



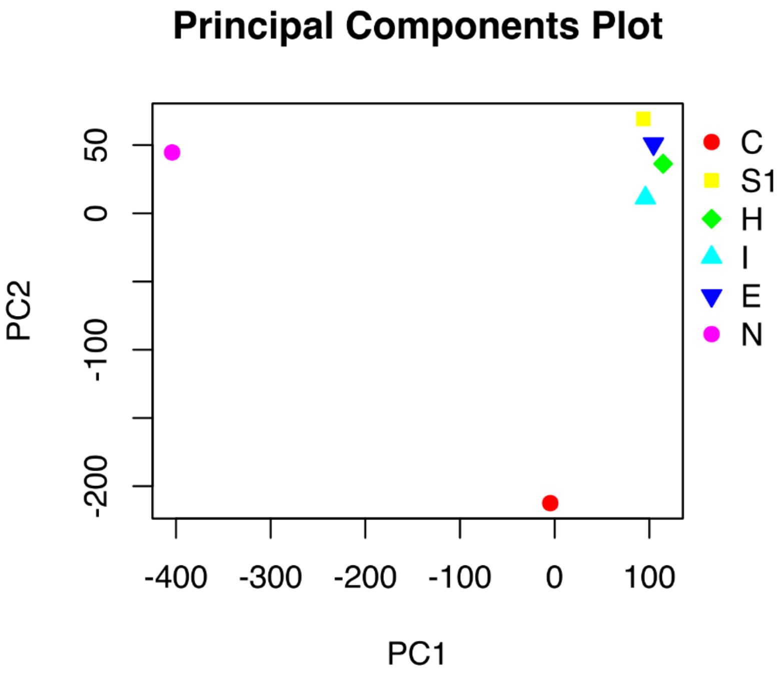
Surgery samples from Georgia

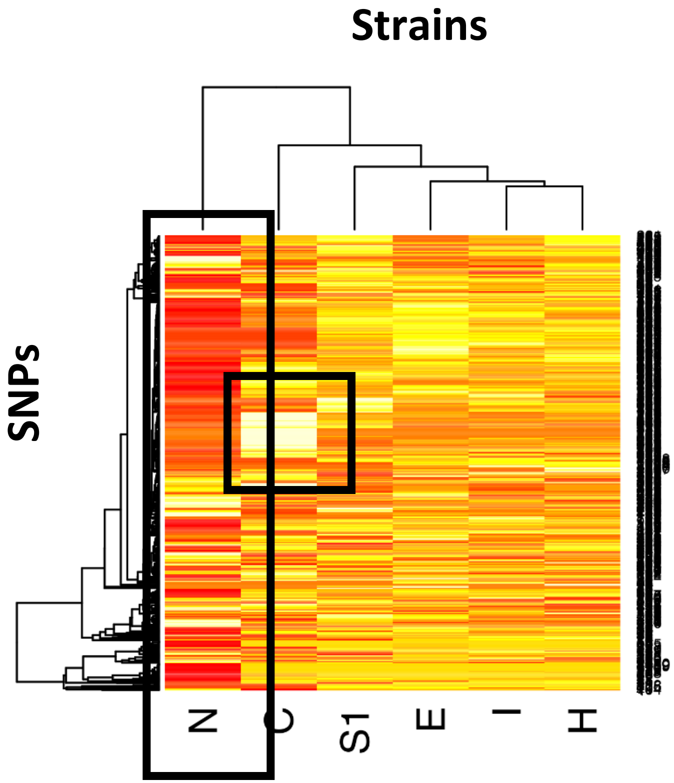
L2.2.1

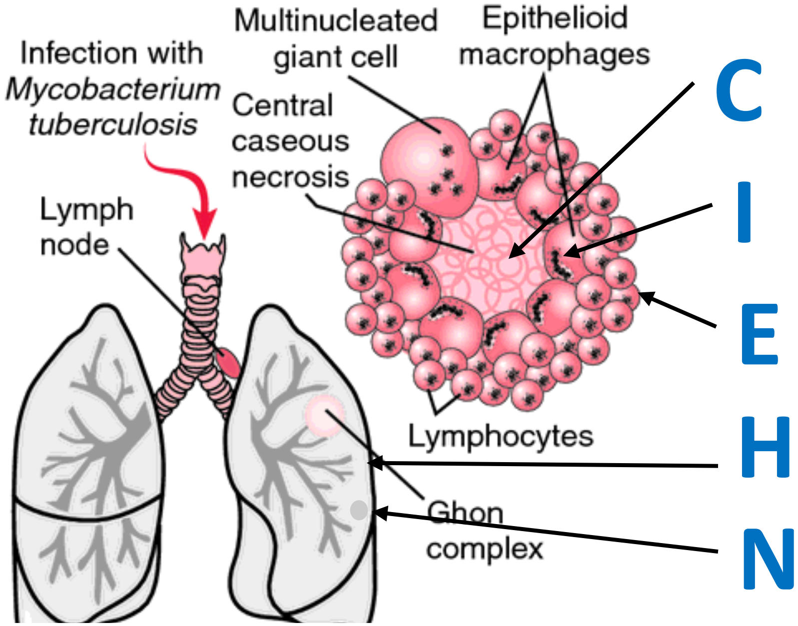


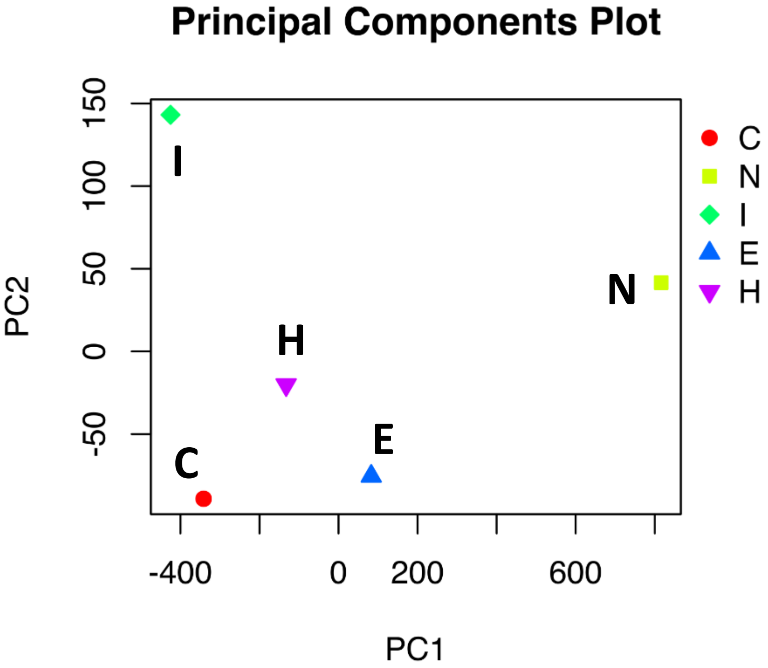
Sample topography

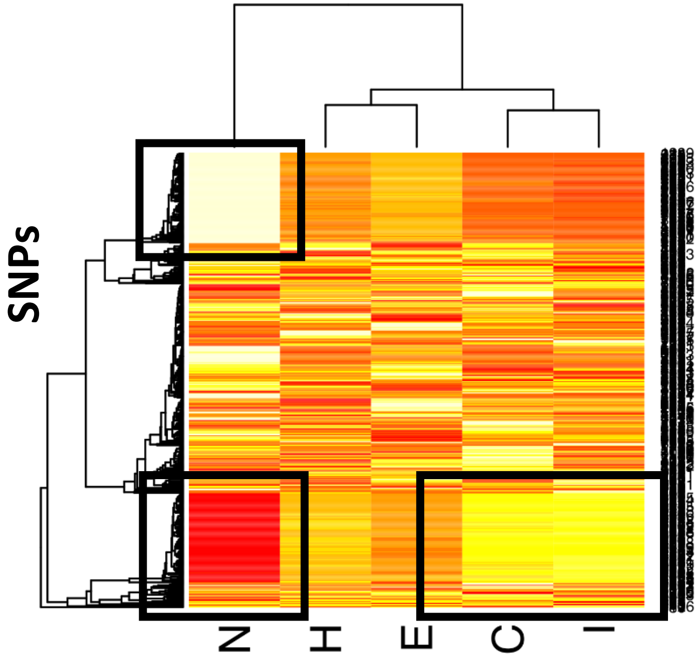
- S 1: diagnostic sputum
- C: cavity of granuloma
- I: inner granuloma wall
- E: external granuloma wall
- H: Healthy tissue
- N: Secondary infectious nodule


Principal Components Plot


Strains

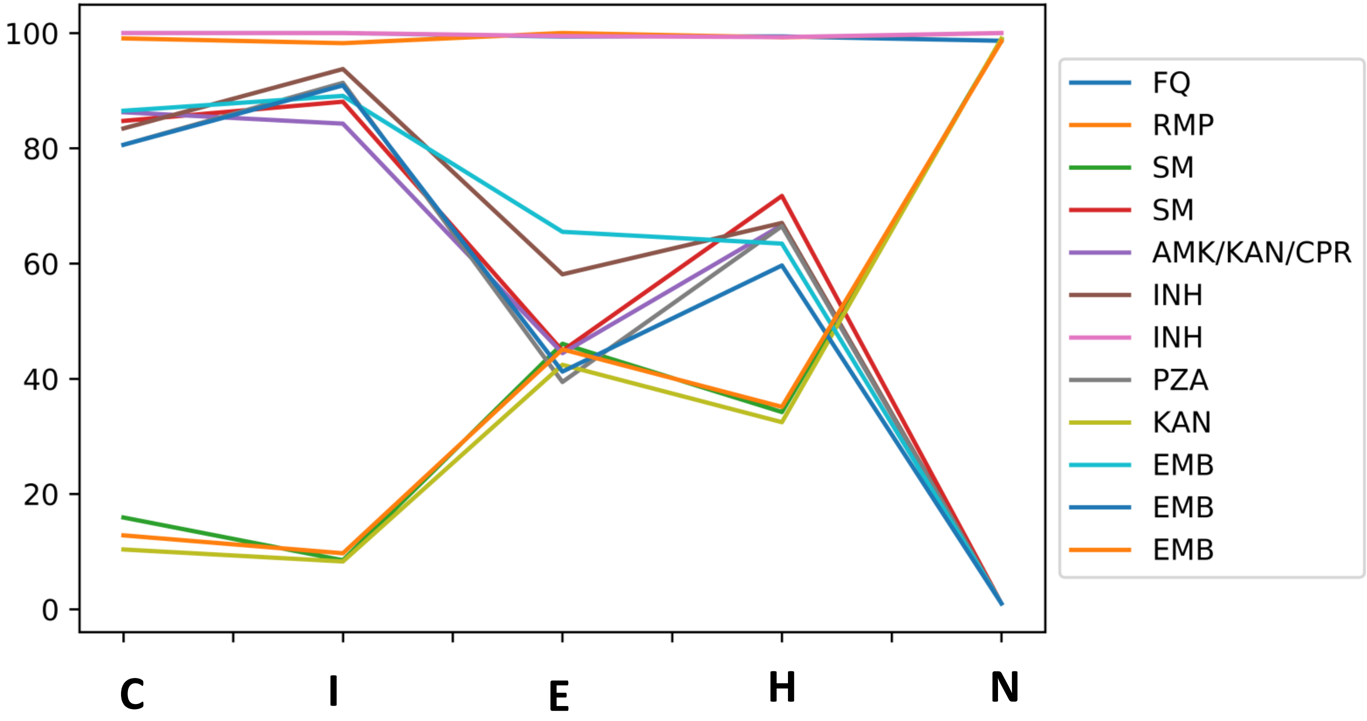




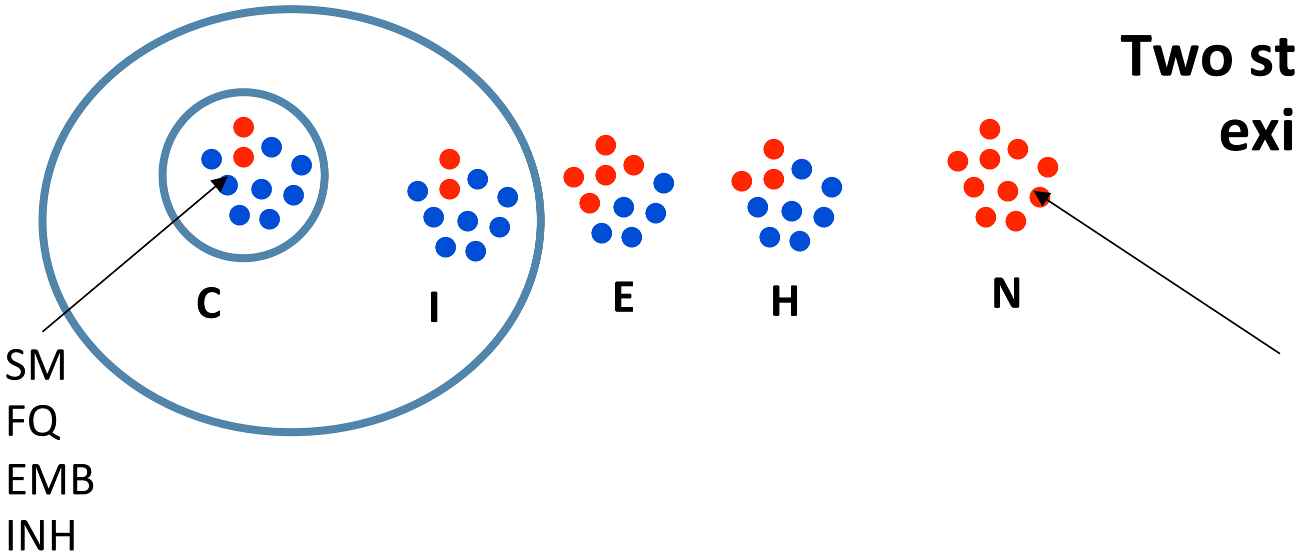


Moreno et al. Unpublished

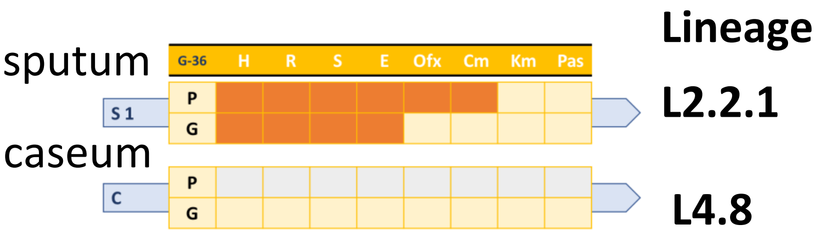
MDR STRAIN (FIXED MUTATIONS TO INH + RIF)



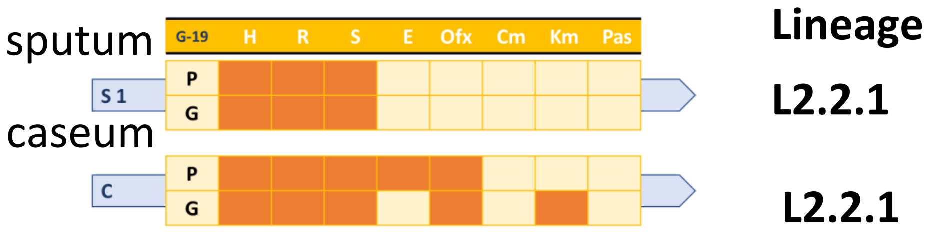
Two strains co-existing



Two cases with co-infection

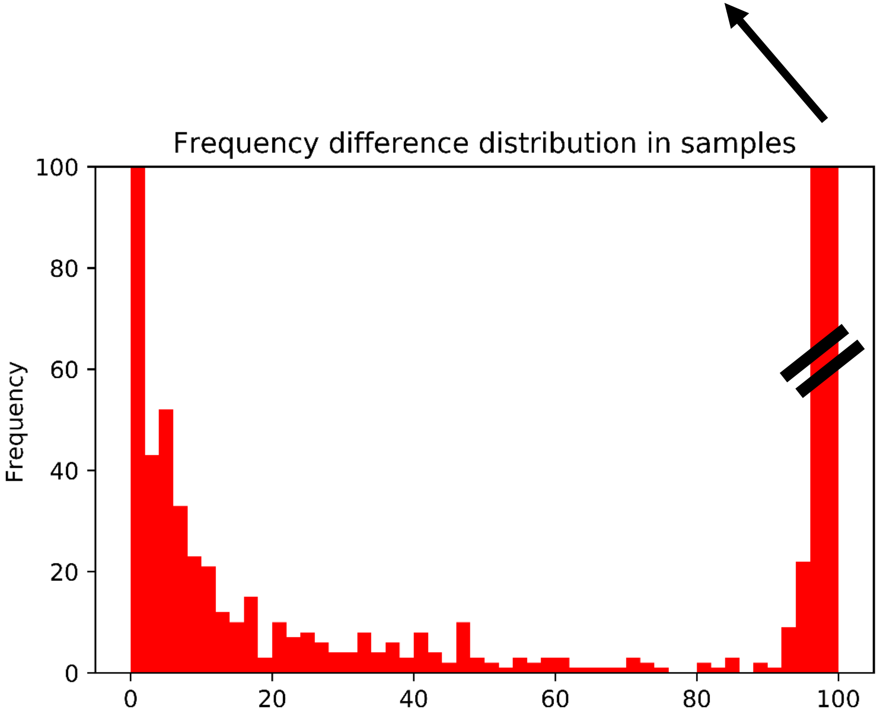


		G-36	H	R	S	E	Ofx	Cm	Km	Pas	Lineage
sputum	S 1	P	🟧	🟧	🟧	🟧	🟧	🟧	⬜	⬜	L2.2.1
		G	🟧	🟧	🟧	🟧	⬜	⬜	⬜	⬜	
caseum	C	P	▨	▨	▨	▨	▨	▨	▨	▨	L4.8
		G	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	

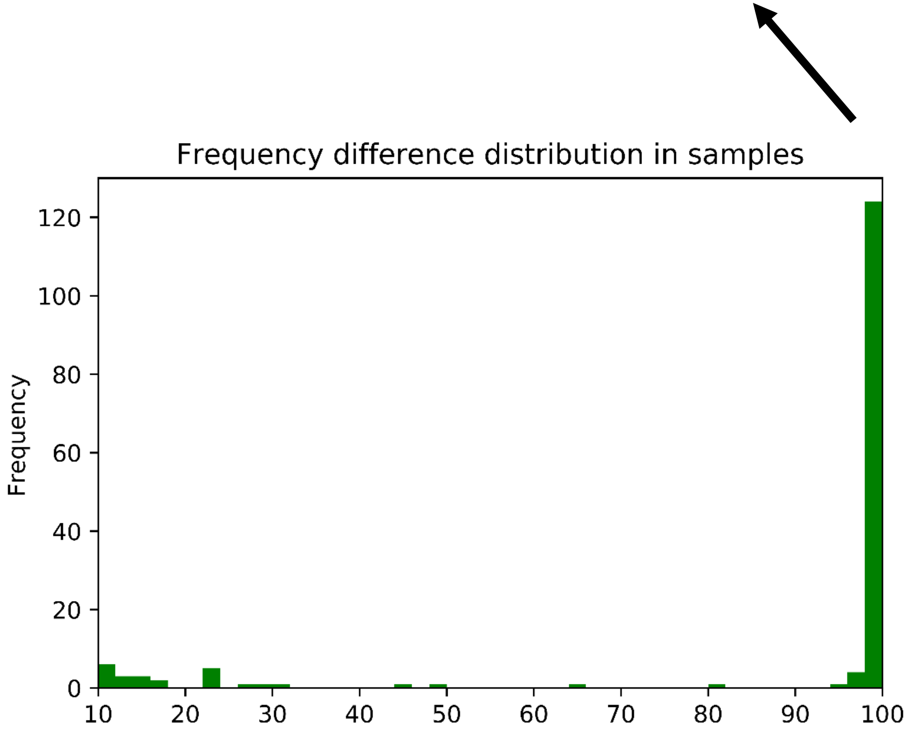


		G-19	H	R	S	E	Ofx	Cm	Km	Pas	Lineage
sputum	S 1	P	🟧	🟧	🟧	⬜	⬜	⬜	⬜	⬜	L2.2.1
		G	🟧	🟧	🟧	⬜	⬜	⬜	⬜	⬜	
caseum	C	P	🟧	🟧	🟧	🟧	🟧	⬜	⬜	⬜	L2.2.1
		G	🟧	🟧	🟧	⬜	🟧	⬜	🟧	⬜	

Number of fixed SNPs apart: 1173

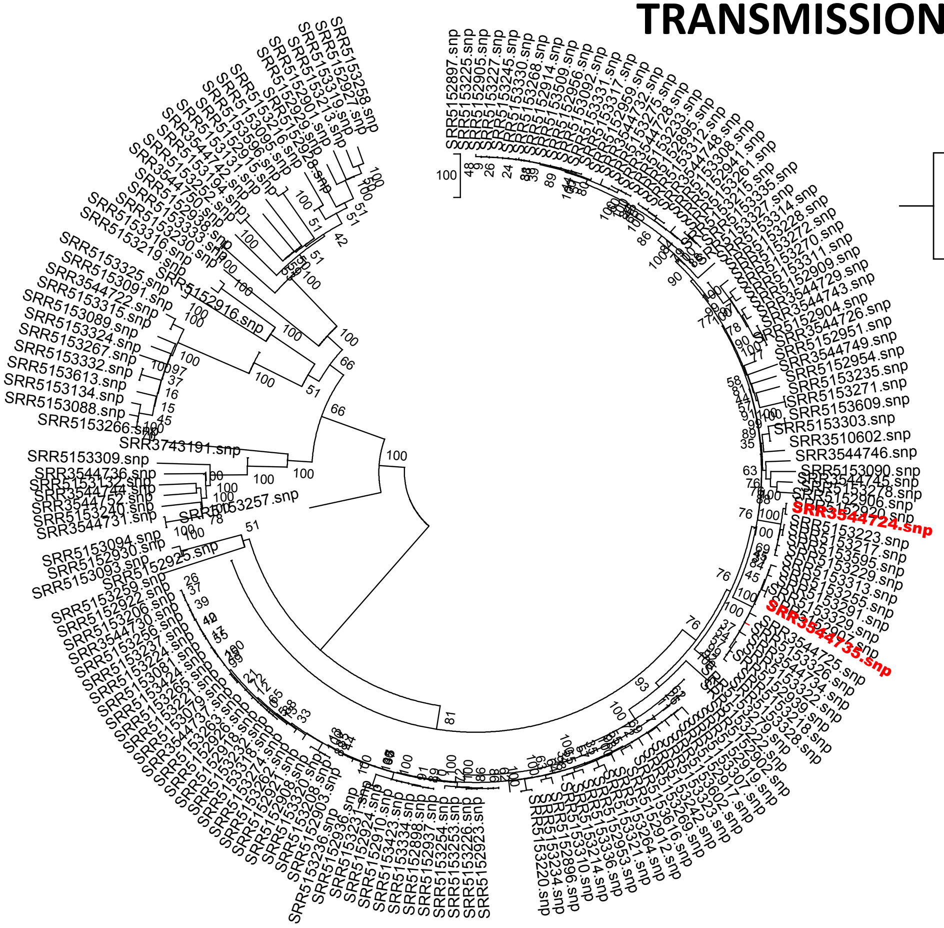


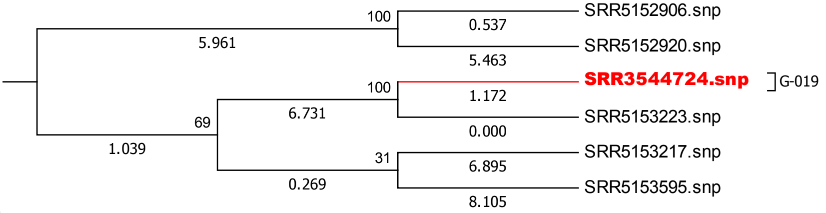
Number of fixed SNPs apart: 124

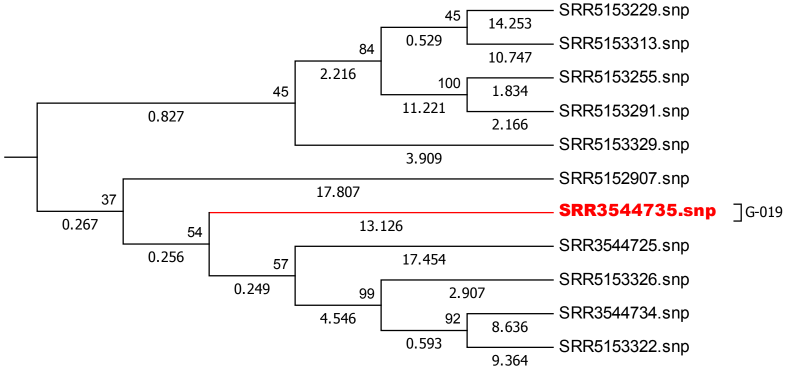


Moreno et al. Unpublished

G-19 CO-INFECTION WITH TWO CLOSELY RELATED STRAINS BELONGING TO TWO DIFFERENT TRANSMISSION CLUSTERS



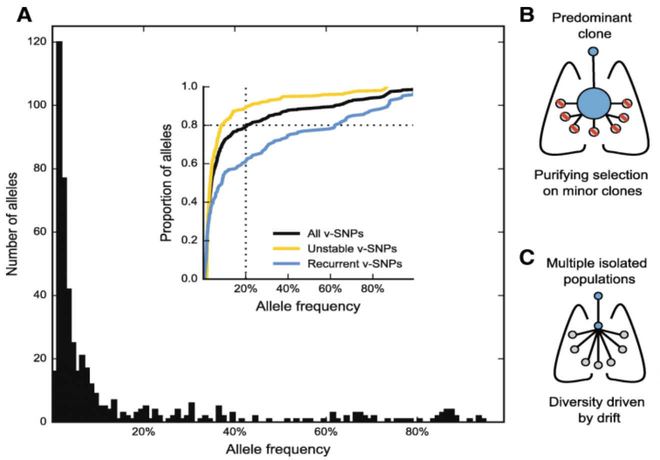




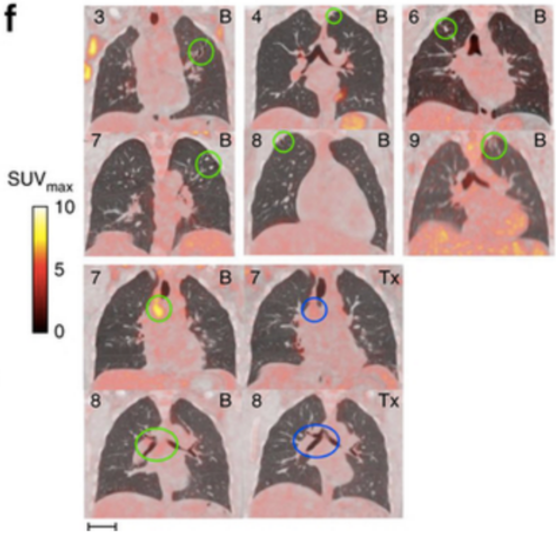
Georgia population structure separates the two strains

Moreno et al. Unpublished

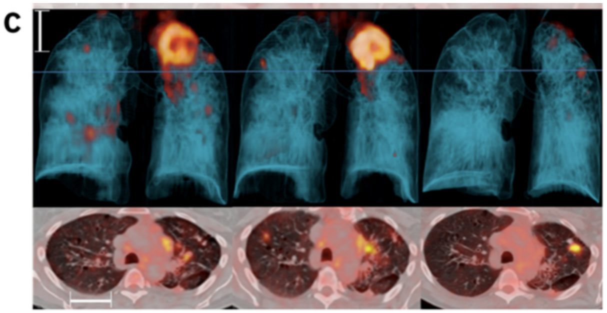
Need to link MTBC population structure, drug penetration, lesion heterogeneity during infection



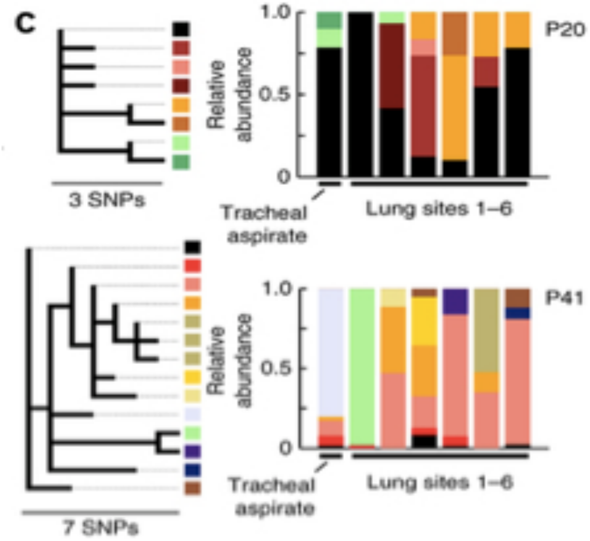
Trauner A. et al. Genome Biology 201



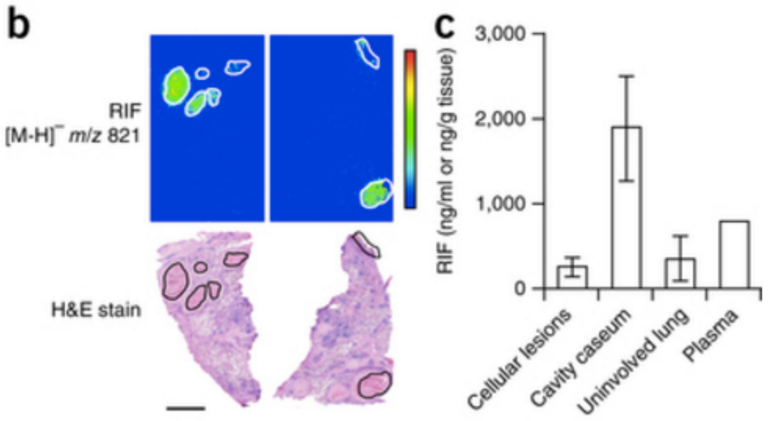
Smail et al. Nat Med 2016



Malherbe et al. Nat Med 2016



Lieberman et al. Nat Med 2016



Prideaux et al. Nat Med 2015

Short-read

Illumina

Pros:
Paired-end reads
Up to 2x300pb
Low error rate

Cons:
Limited structural information
GC-content bias
Reference mapping

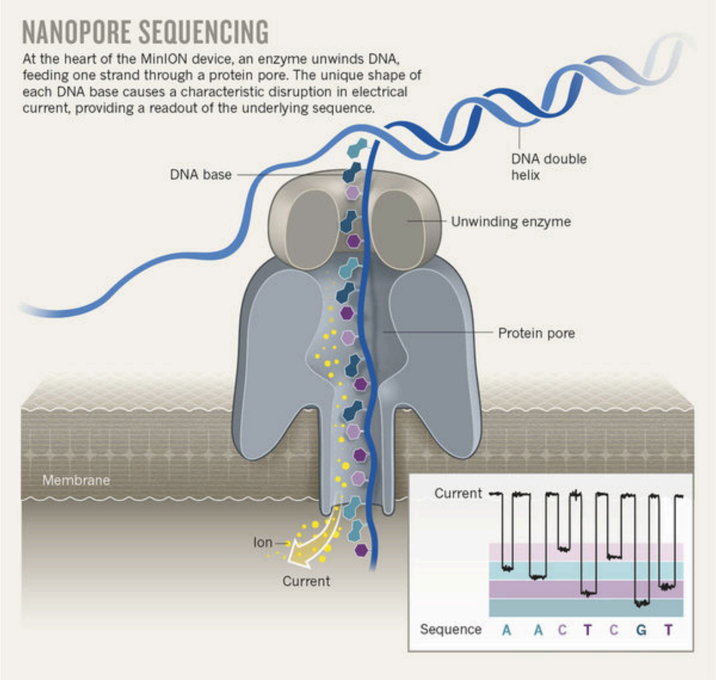
Long-read

PacBio
Nanopore

Pros:
High structural information
‘Real time’ sequencing
‘No’ GC-bias
De-novo assembly

Cons:
high base errors ~11-15%
accuracy

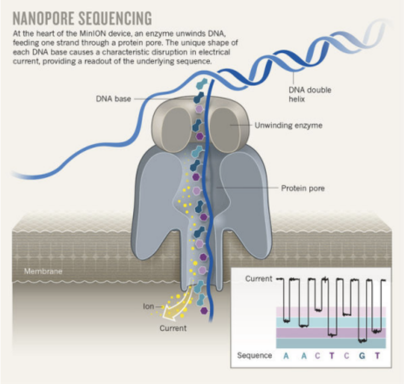
- Oxford Nanopore Technology:


NANOPORE SEQUENCING

At the heart of the MinION device, an enzyme unwinds DNA, feeding one strand through a protein pore. The unique shape of each DNA base causes a characteristic disruption in electrical current, providing a readout of the underlying sequence.

- Run in <48H
- No PCR amplification
- Possibility of Real Time calling

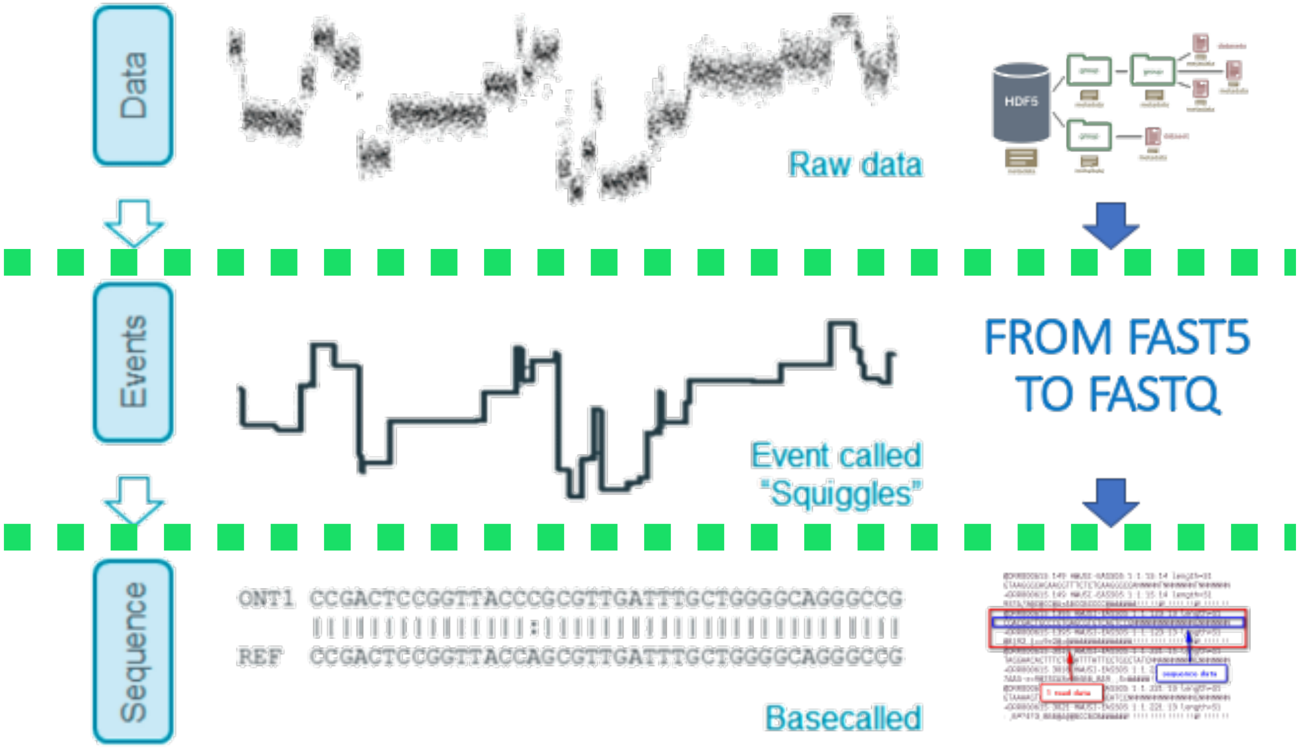
- Oxford Nanopore Technology:



NANOPORE SEQUENCING

At the heart of the MinION device, an enzyme unwinds DNA, feeding one strand through a protein pore. The unique shape of each DNA base causes a characteristic disruption in electrical current, providing a readout of the underlying sequence.

Based on changes in electric current density when occupied by DNA/RNA



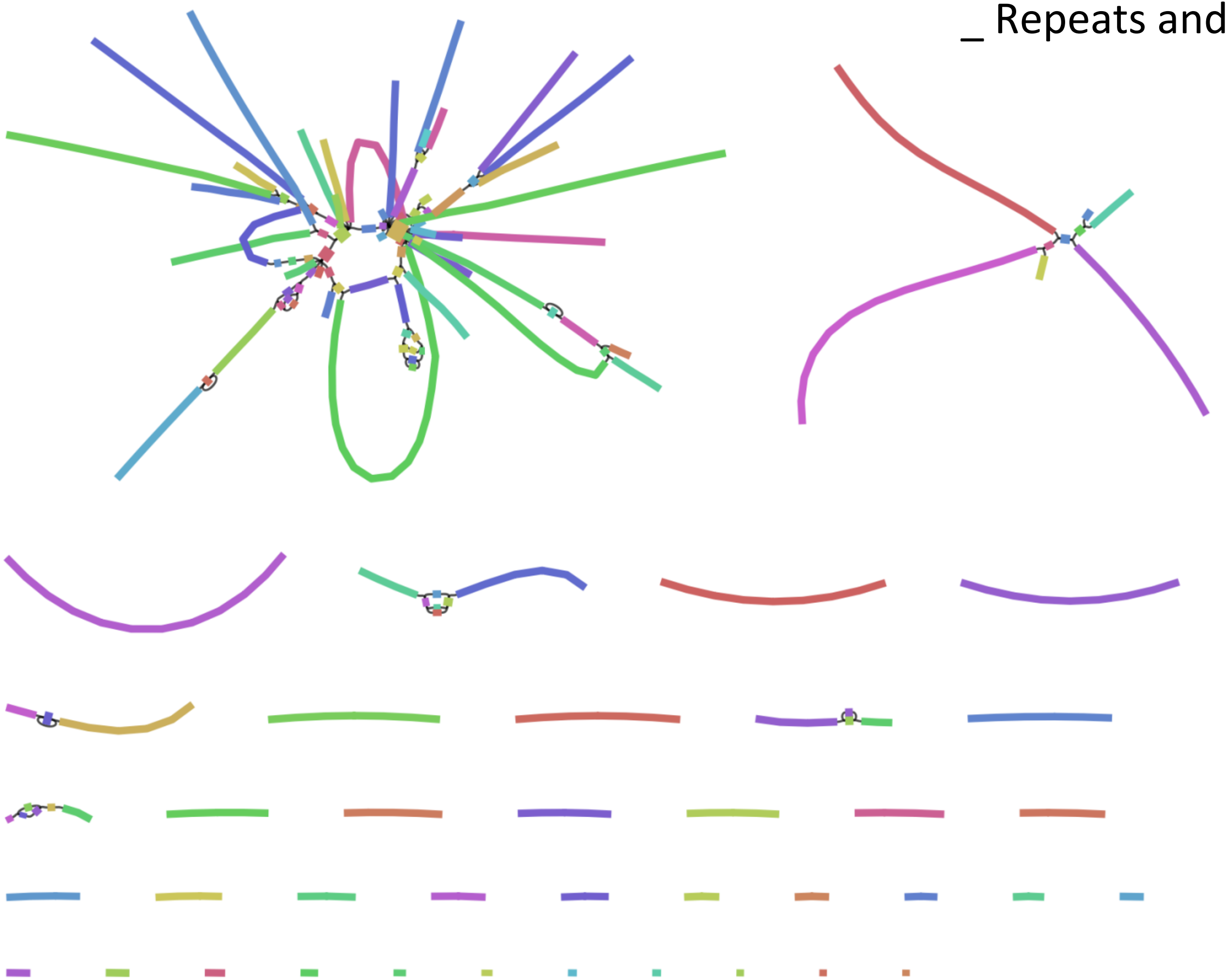
```
ONT1 CCGACTCCGGTTACCCGCGTTGATTTGCTGGGGCAGGGCCG
     |||||||||||||||:|||||||||||||||||||||||||
REF  CCGACTCCGGTTACCAGCGTTGATTTGCTGGGGCAGGGCCG
```

Stochastic errors

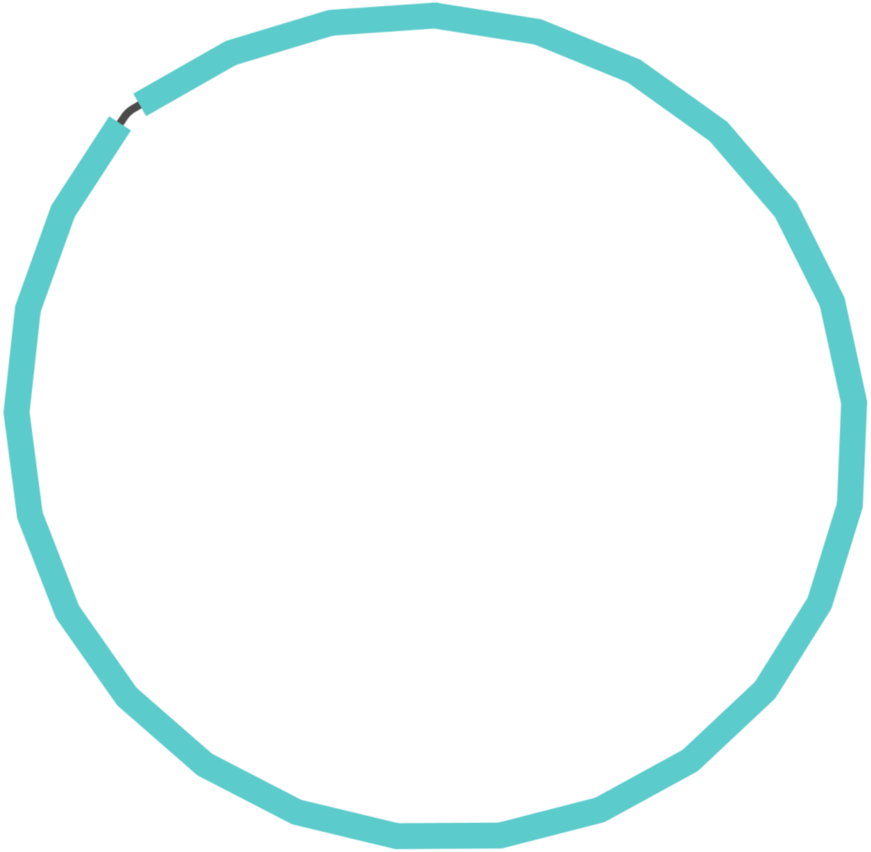
Systematic errors

RESULTS G990

Short-read de novo assembly (MiSeq):
_120 contigs
_ Repeats and complex regions not solved



RESULTS G990



Long read nanopore de novo:

1 closed contig

Reads 423,401

Depth cov 210

Mean read lenght 1,760

Longest read 498,093

We can look at:

1. Variation in PE/PPE
2. Position of IS6110
3. Insertions/deletions
4. Duplications
5. Other repetitive regions

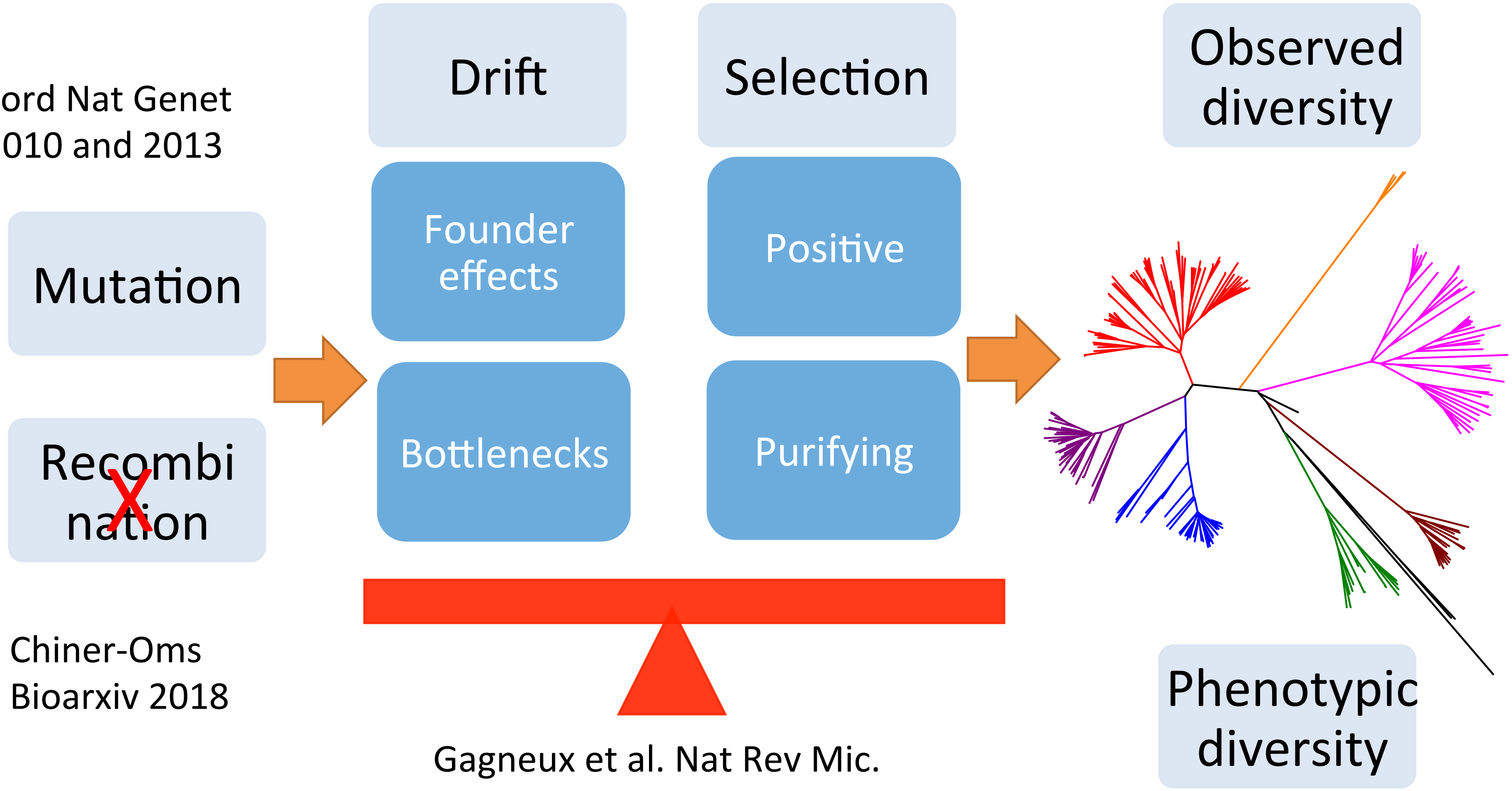
Studies involving WGS

Hersbergh Plos Biology 2008

Osorio MBE 2013, Peperell Plos Pathogens 2013

Lee Rs PNAS 2015, Pepperell PNAS 2011, Merker, Nat Genet 2015, Casali 2014

Ford Nat Genet 2010 and 2013



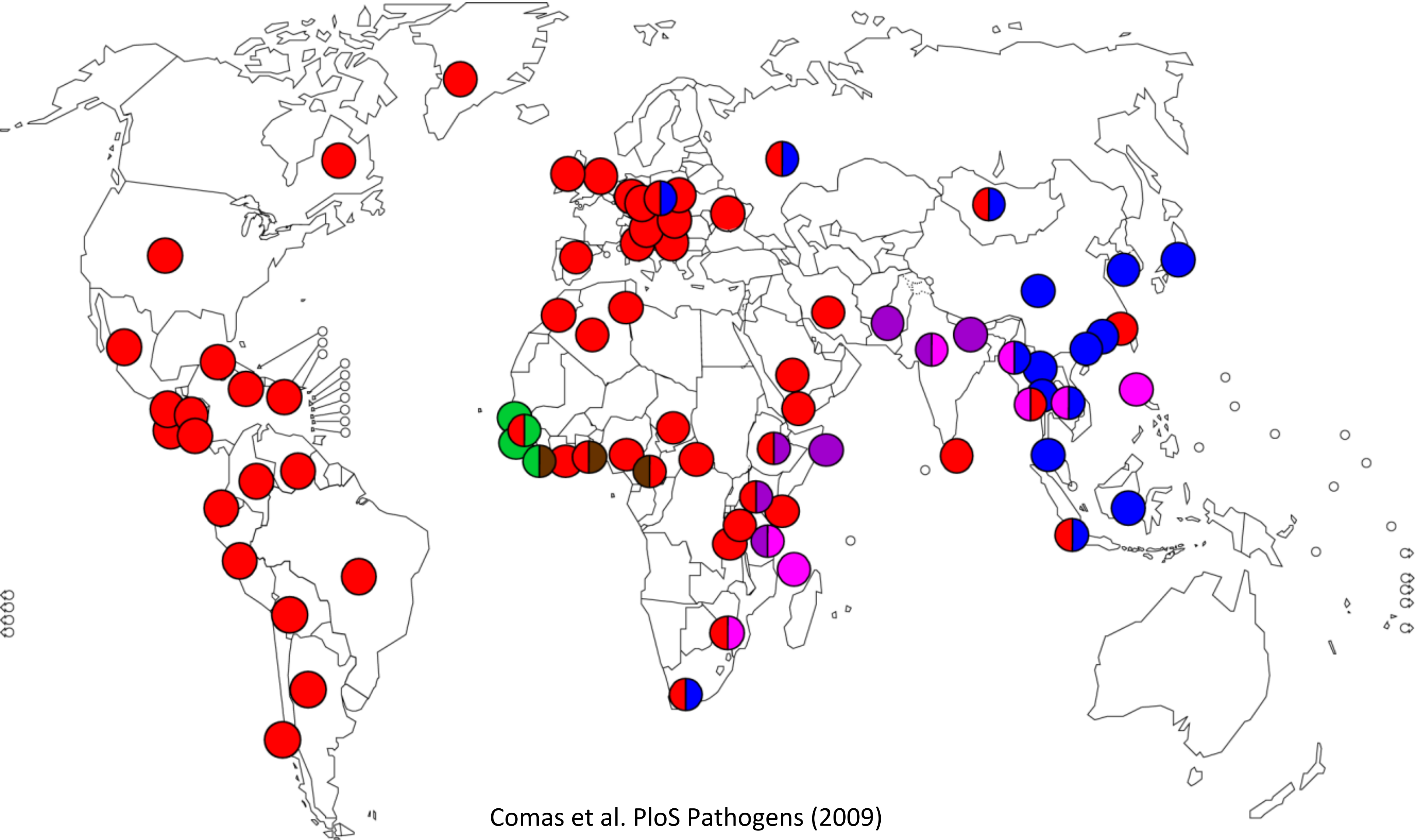
Chiner-Oms Bioarxiv 2018

Gagneux et al. Nat Rev Mic. 2018

TAKE HOME MESSAGES

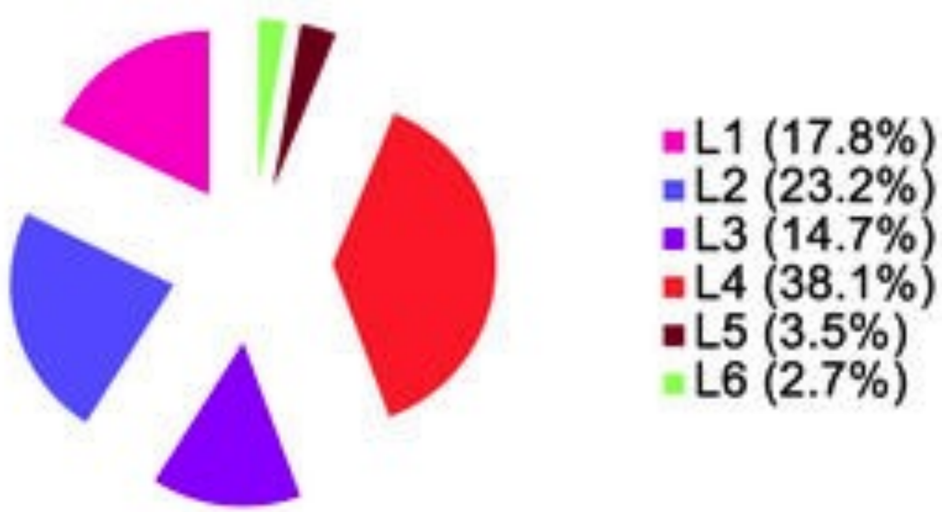
- Hallmarks of selective pressure can now be detected using thousands of strains
- High resolution to reveal global and local diversity patterns
- Impact of lineage genetic background but also of specific genotypes in transmission
- Genomic diversity within host limited by sputum-based analyses – mostly studied for DR
- Long read sequencing technologies will lead to shift in MTBC genomic analyses

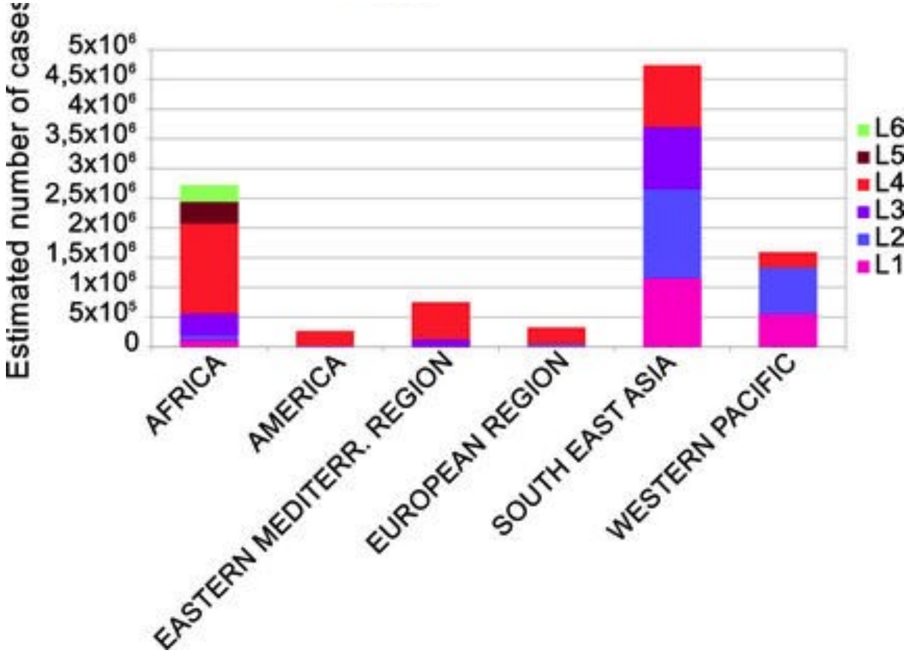
(human) MTBC global diversity



Comas et al. PloS Pathogens (2009)

Lineage 4 causes up to 38% of TB cases and is present in all regions





Chiner-Oms Scientific Reports
2018

Less than 2,500 genetic variants can explain striking differences in biology

- Differences in host ranges
- Transmission dynamics
- Drug resistance
- Immune responses
- Latency
- …etc

- We should be able to easily identify causal variants in MTBC

- We are only achieving incremental progress

e.g Coll 2018 -> 6,465 isolates

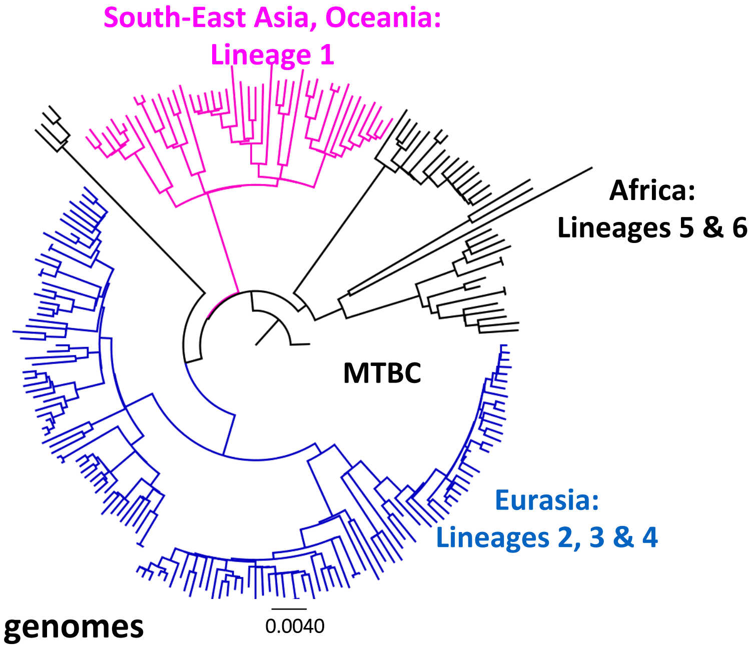
- Far from identifying drug resistance genetic architectures

e.g see Hicks Nat Micro 2018 and Colangeli NEJM 2018

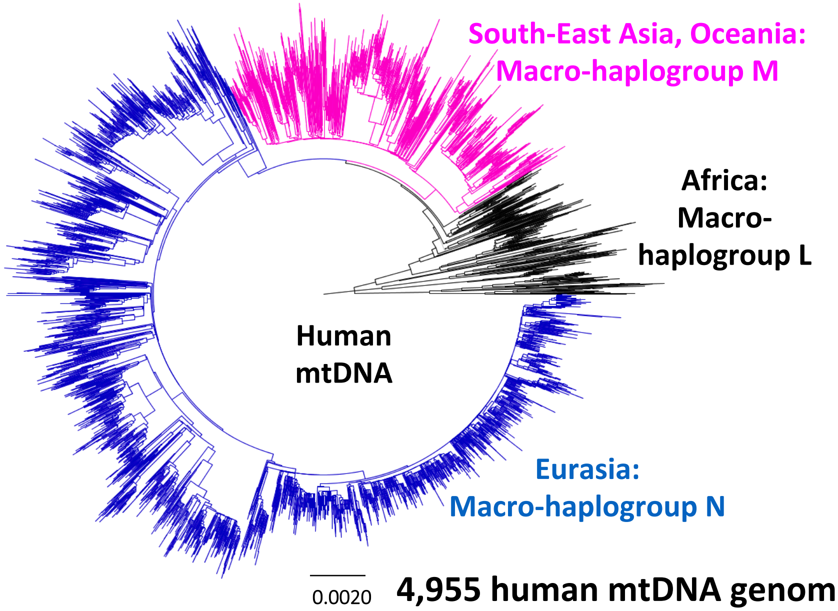
- Very far from identifying relevant virulence variants

but see Asensio PNAS 2014

Strong parallels between human and MTBC phylogenies



220 MTBC genomes



4,955 human mtDNA genomes

TB 20th century: a story of neglect

- **No R&D**
- **Public health infrastructure dismantled**
- **No surveillance**

1900

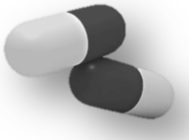
1950

TB eradication is assumed

2000

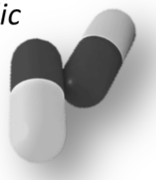
1900

Sputum smear microscopy
PPD test



1920
BCG vaccine

1943-1946
Streptomycin
Para-aminosalisylic acid (PAS)

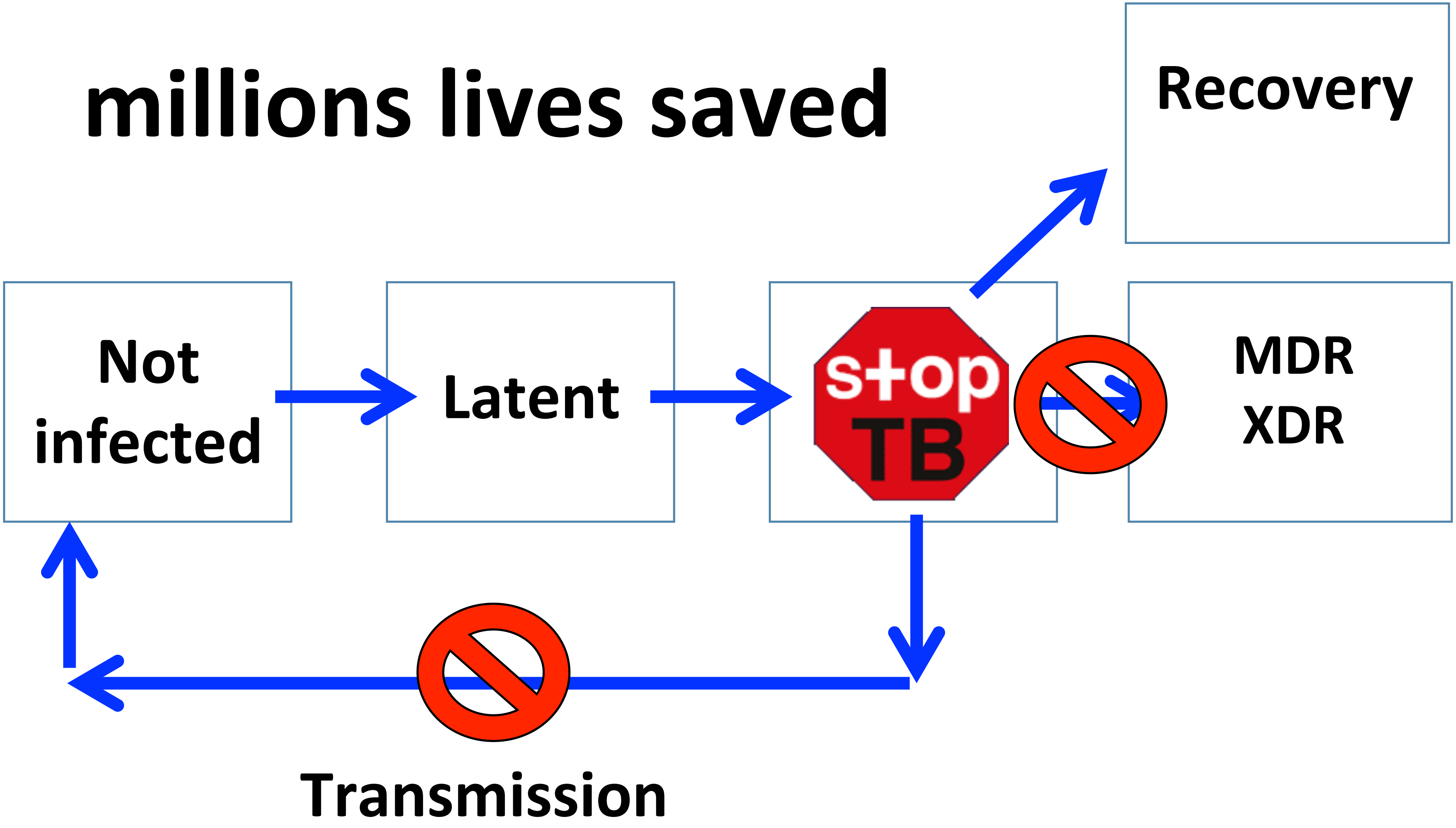


1952-1962
Isoniazid
Pyrazinamide (PZA)
Cycloserine
Ethambutol
Rifampicin

1993
WHO Global Health Emergency
8 Million cases
2.5 Million Deaths

DOTS

DOTS: Directly Observed Treatment, Short-Course



millions lives saved

Recovery

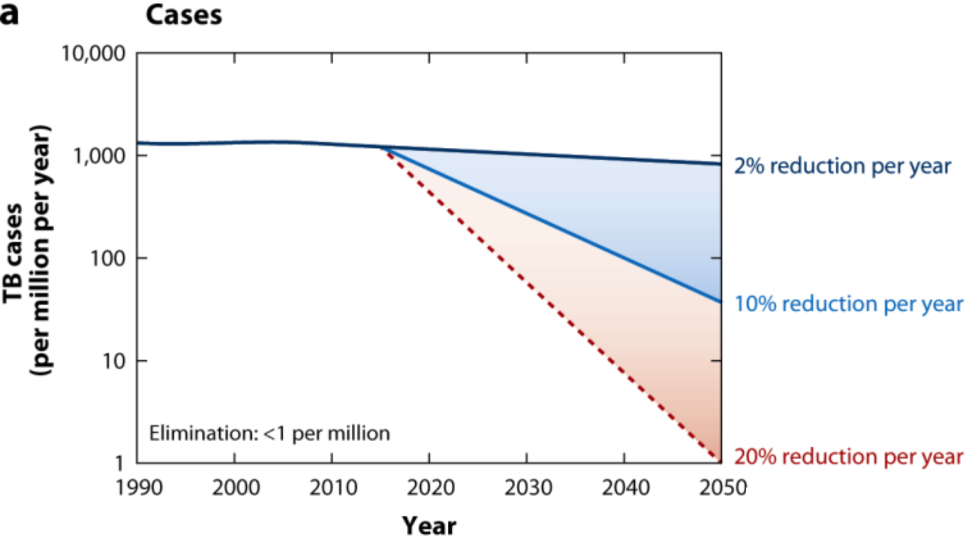
Not infected

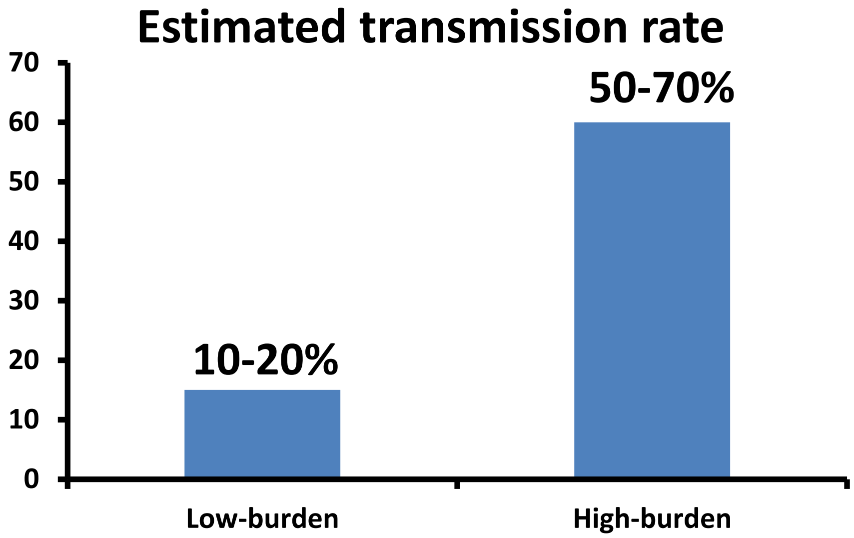
Latent

MDR
XDR

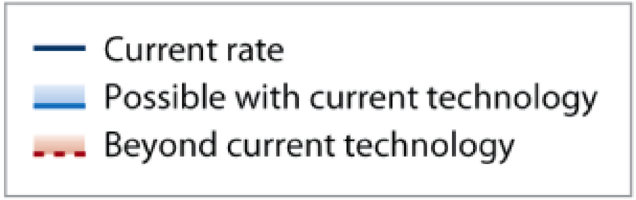
Transmission

Slow and Steady is not an option!





Dye 2013



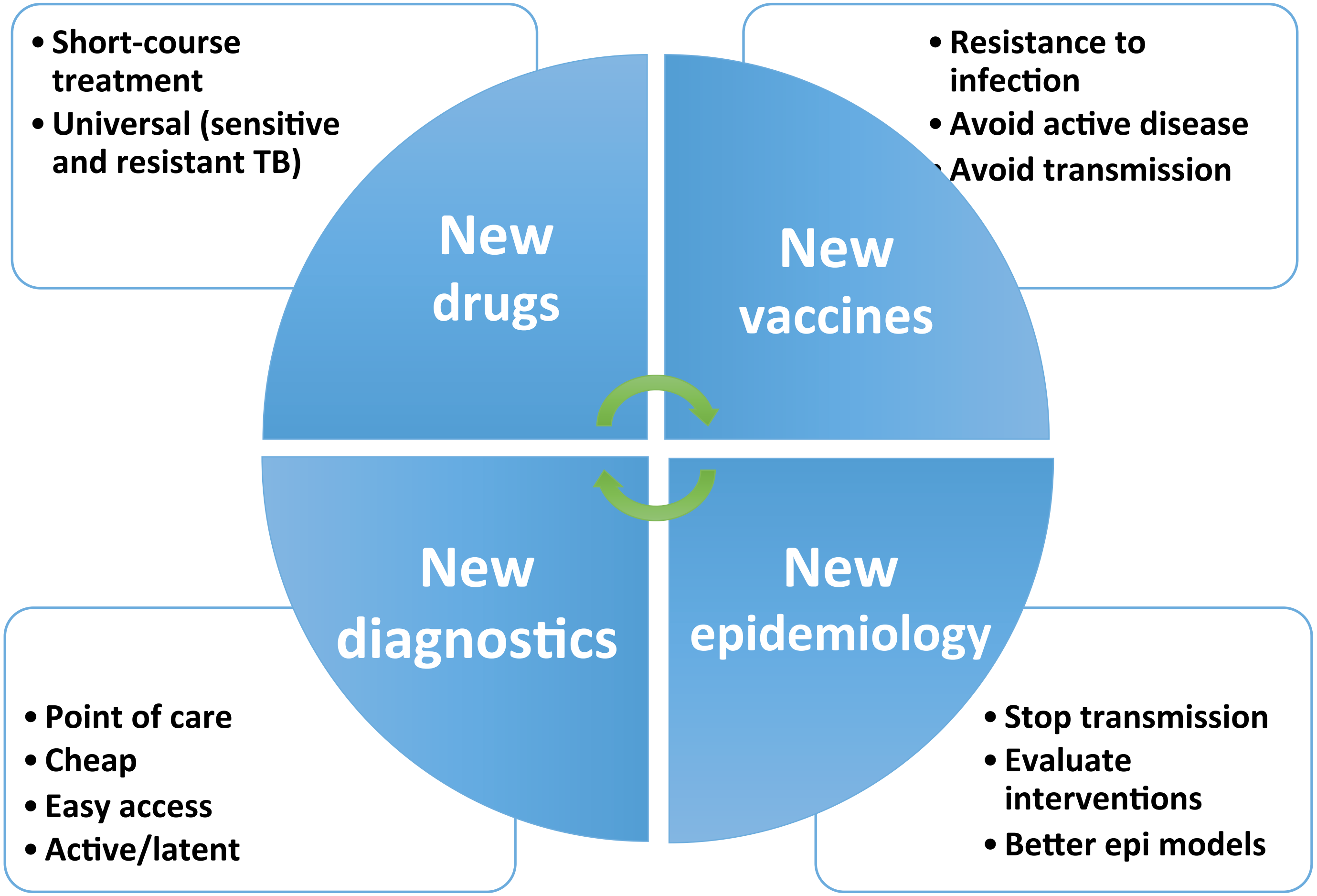
- **Decades old tools**
- **Inaccurate epidemiological models**
- **Knowledge gaps on host-pathogen**

- **Transmission is on-going!**
- **Contributes to latent reservoir -> future epidemics**

Modernizing DOTS to accelerate TB eradication

2013 – Bedaquiline/Delamanid

2013 – MVA85A



New drugs

- **Short-course treatment**
- **Universal (sensitive and resistant TB)**

New vaccines

- **Resistance to infection**
- **Avoid active disease**
- **Avoid transmission**

New diagnostics

- **Point of care**
- **Cheap**
- **Easy access**
- **Active/latent**

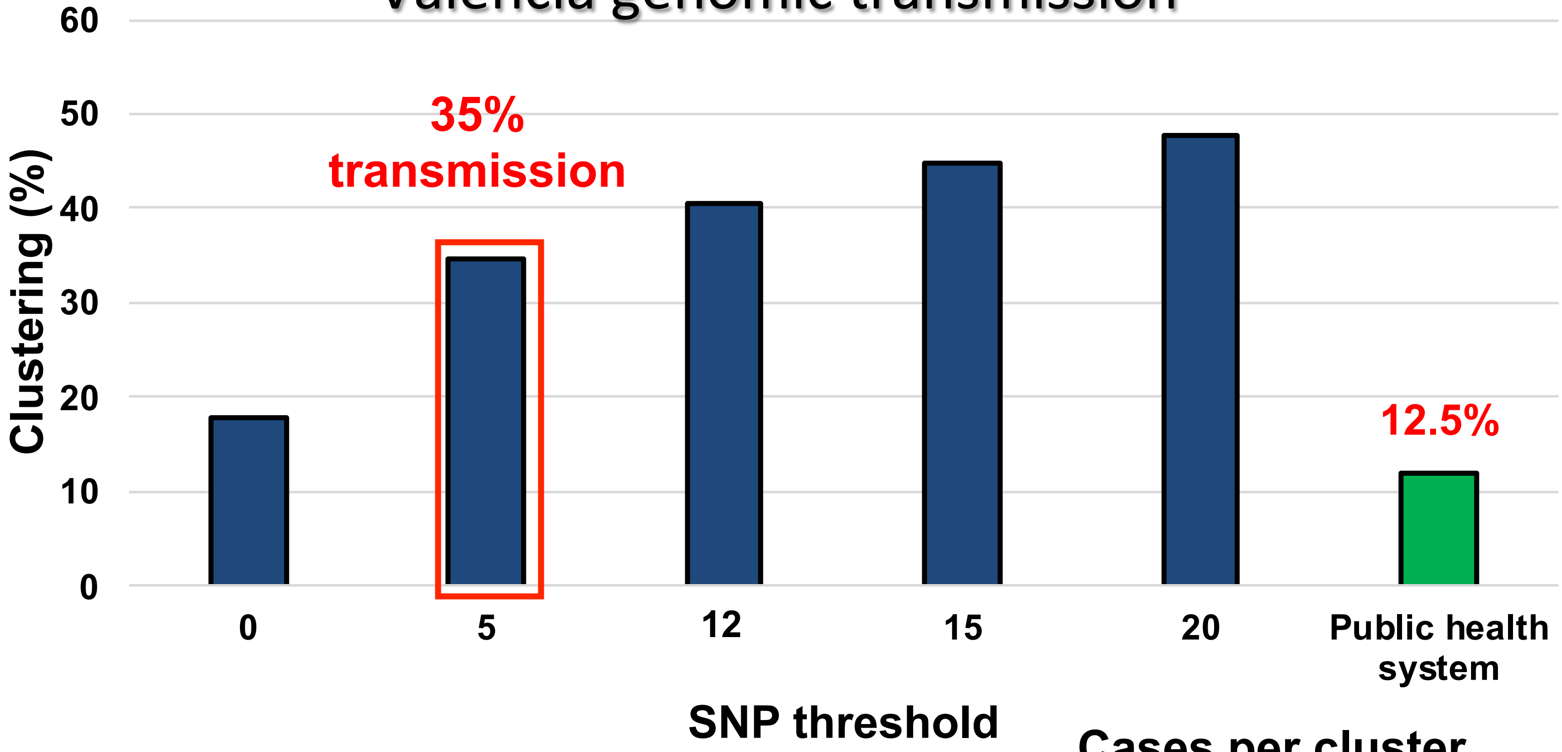
New epidemiology

- **Stop transmission**
- **Evaluate interventions**
- **Better epi models**

2010 - Expert MTB/RIF

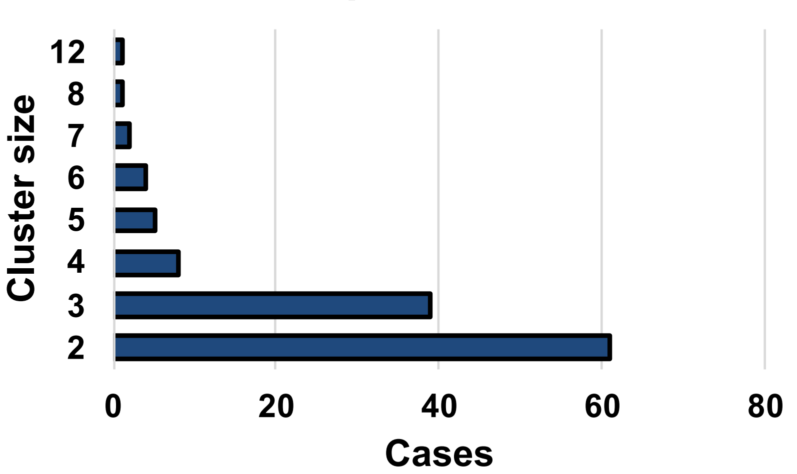
2010- Whole genome

Valencia genomic transmission



121 transmission clusters

Cases per cluster



Cancino et al. Unpublished

Current recommended six months treatment for drug susceptible TB:

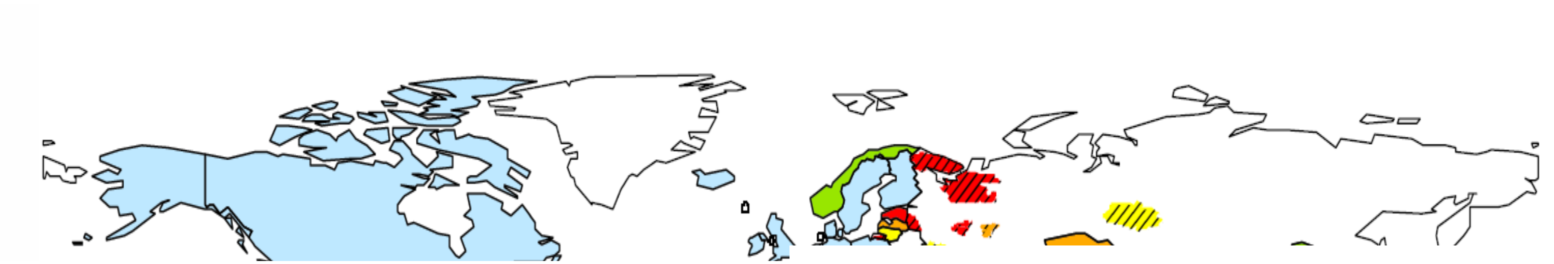
Months	Drugs			
2	Rifampicin	Isonazid	Pyrazynamide	Ethambutol
4	Rifampicin	Isonazid		

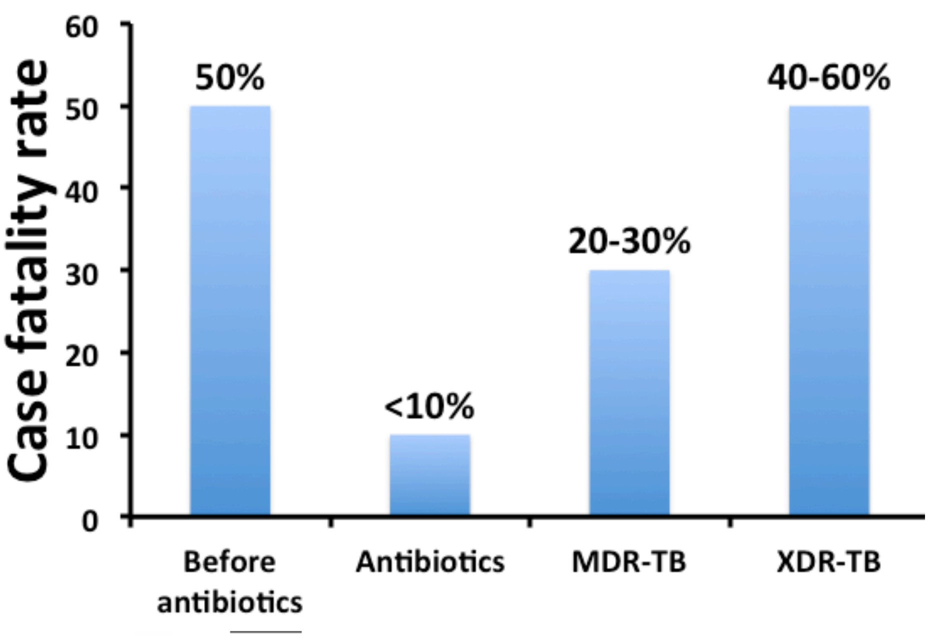
Definition of Multiple- (MDR) and Extensive- (XDR) Drug resistant TB strains

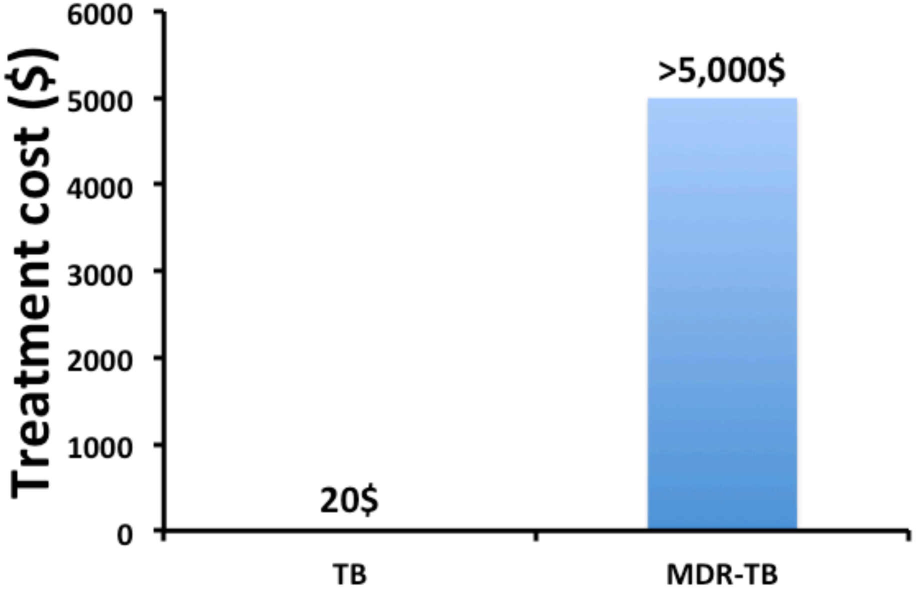
DR TB AS DEFINED BY WHO:	**FIRST LINE DRUGS**		**FLUOROQUINOLONES**	**SECOND LINE DRUGS (injectable)**	**THIRD LINE DRUGS**
	Rifampicin	Isonazid			
MDR-TB	**X**	**X**			
XDR-TB	**X**	**X**	**any**	**>=1**	**Delamanid/ Bedauiline**

Can MDR strains outcompete susceptible strains?

How well can we diagnose drug resistance?







Percentage of MDR cases among new TB cases