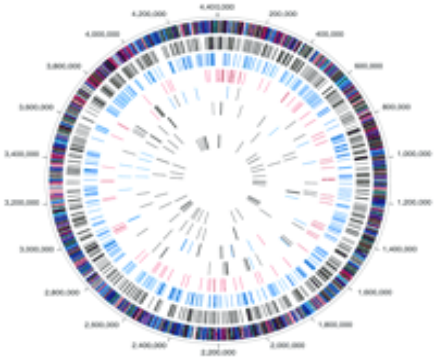
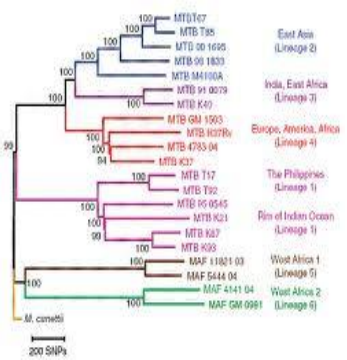
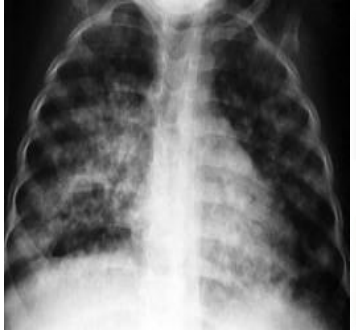
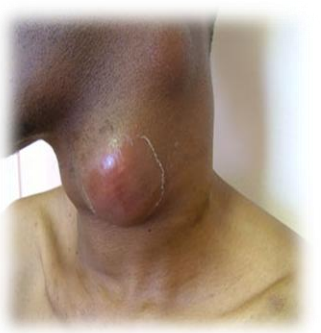
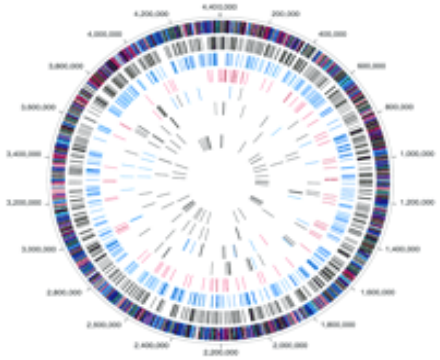
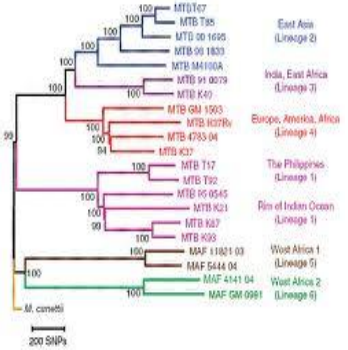
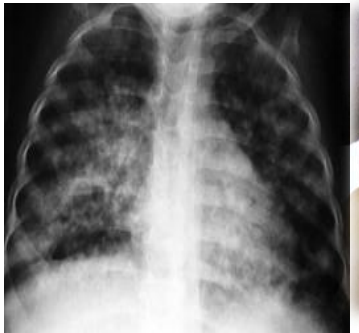
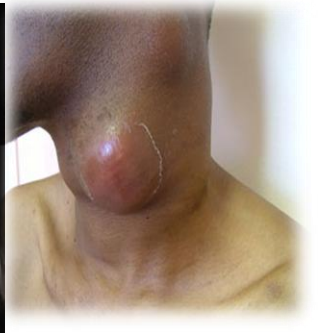




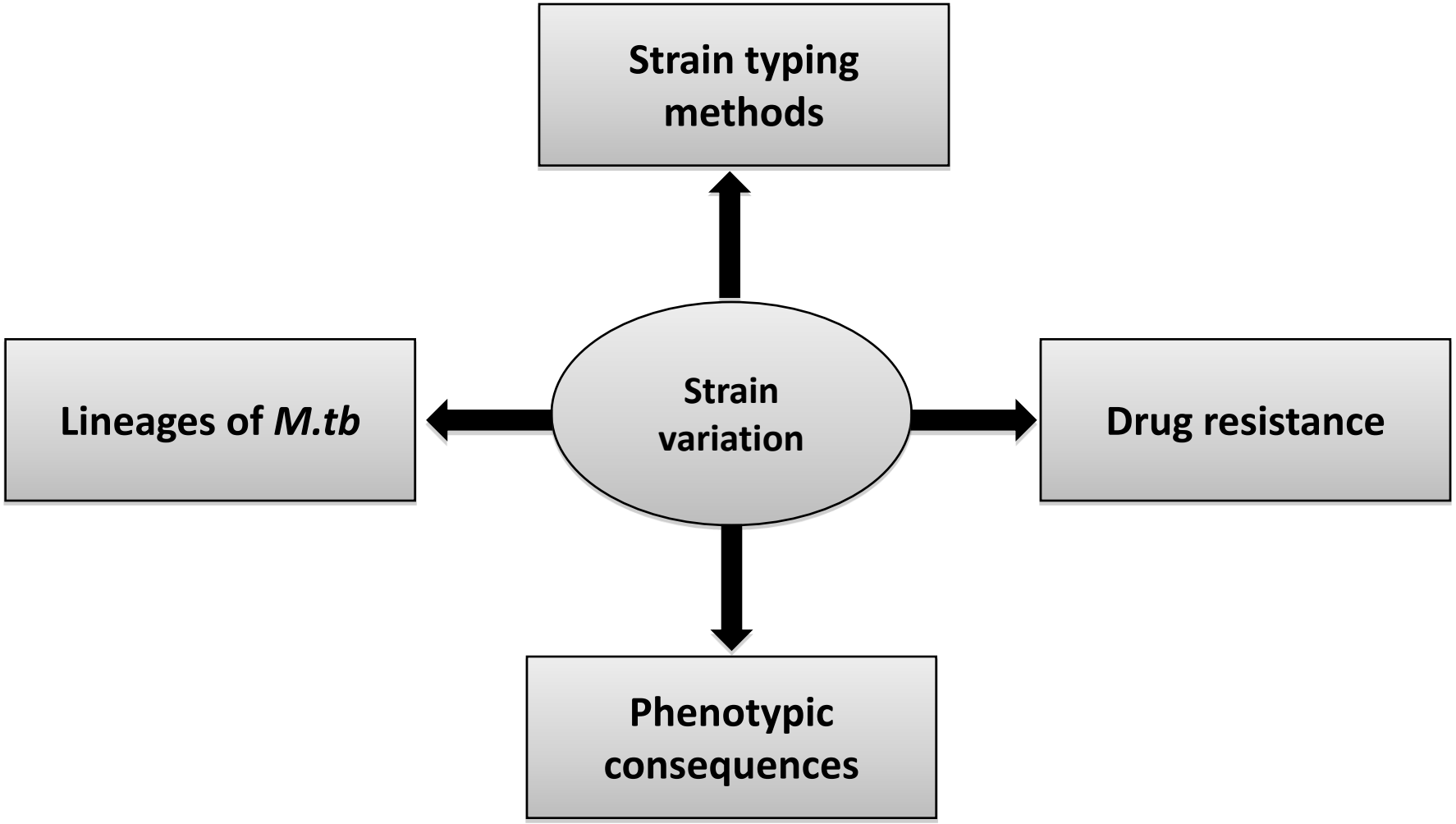
Strain diversity in *Mycobacterium tuberculosis*

Dr. Nitya Krishnan

Overview


Strain typing methods
Lineages of *M.tb*
Strain variation
Drug resistance
Phenotypic consequences

Strain variation

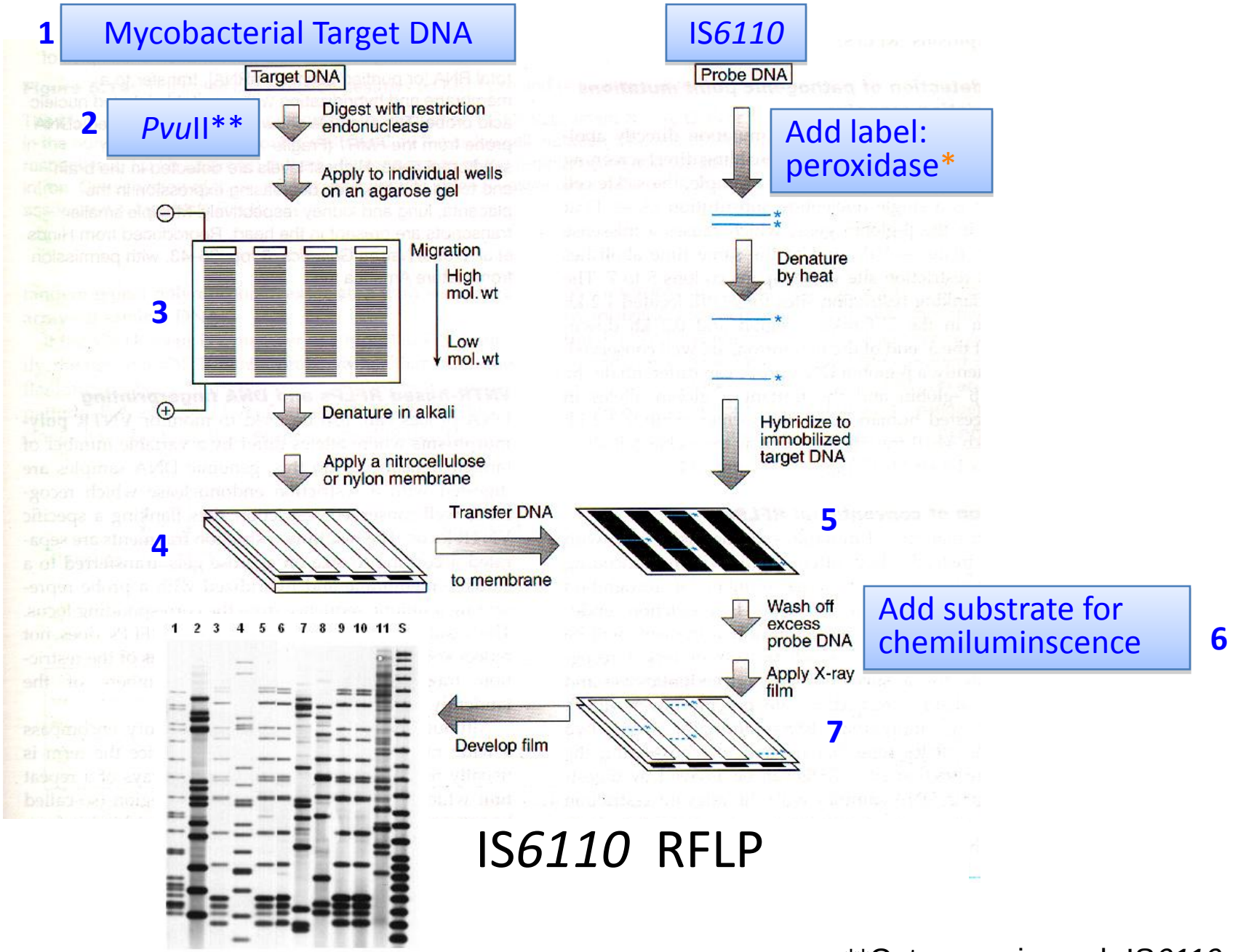
- *M.tb* strains exhibit limited DNA sequence diversity
- Significant genetic diversity generated through recombination, duplication and deletion events
- Highly clonal genetic population structure
- Chromosomal mutations occur in all bacteria
- Horizontal gene transfer is rare

Molecular typing methods

- RFLP
  - Restriction fragment length polymorphism
  - Southern blotting
- Spoligotyping
  - Spacer oligonucleotide typing
  - PCR & hybridization
- Deletion based typing
  - PCR & electrophoresis
- Sequence-based
  - Single Nucleotide Polymorphisms

IS*6110* RFLP analysis

- IS*6110* is a transposable element
- RFLP detects variations in genome caused by IS*6110* insertion
  - **Number** of copies of IS*6110* in chromosome
    - 0-20 copies
  - **Position** of IS*6110* in the bacterial chromosome
    - Size of IS*6110*-containing fragment (RFLP)
- These features are used to discriminate between strains
- I6*110* insertion can occur in both coding (phospholipases, PPE/PE-PGRS families of genes) and non-coding regions

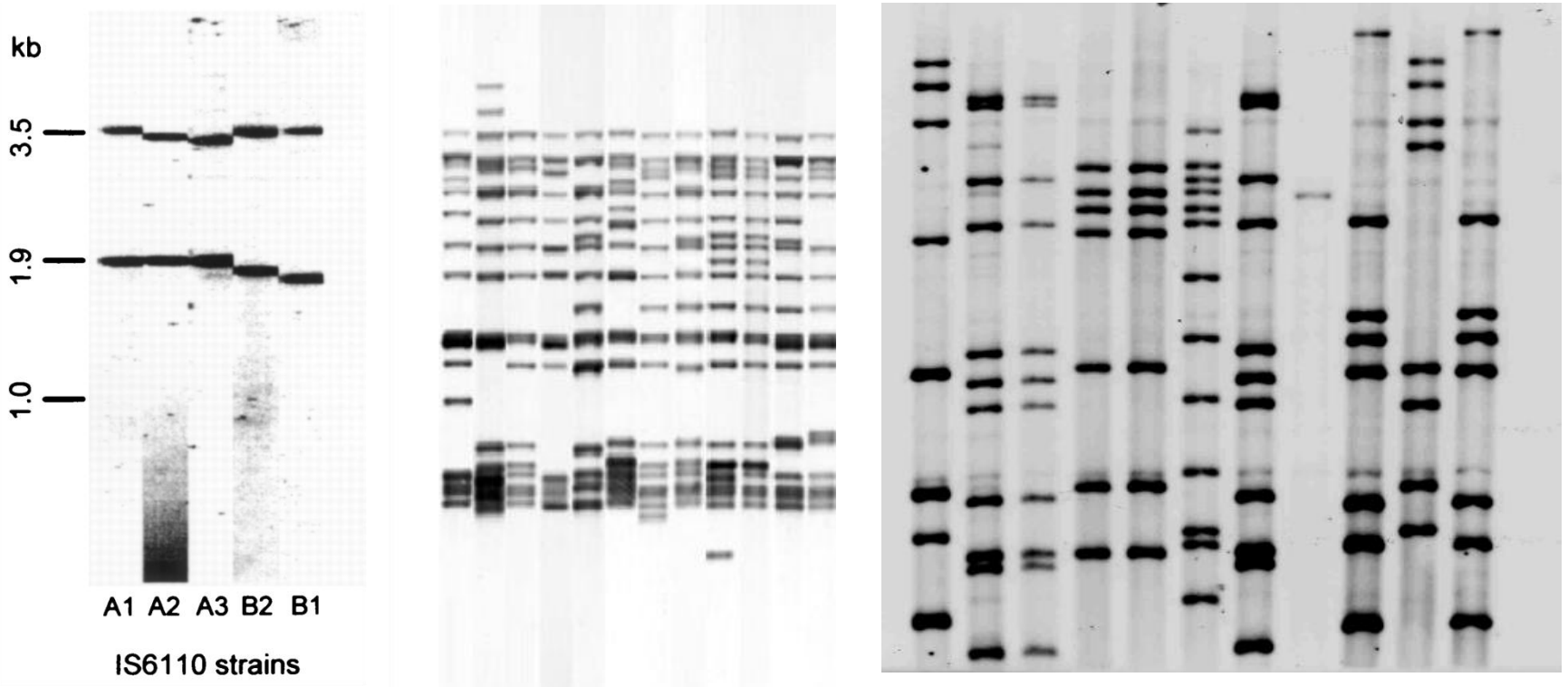


IS*6110* RFLP

**Cuts once in each IS*6110* and throughout chromosome

Example- IS*6110* RFLPs

M. bovis ***M. tuberculosis***



≥7 bands - good discrimination
≤6 bands - poor discrimination

Summary – IS6110 RFLP

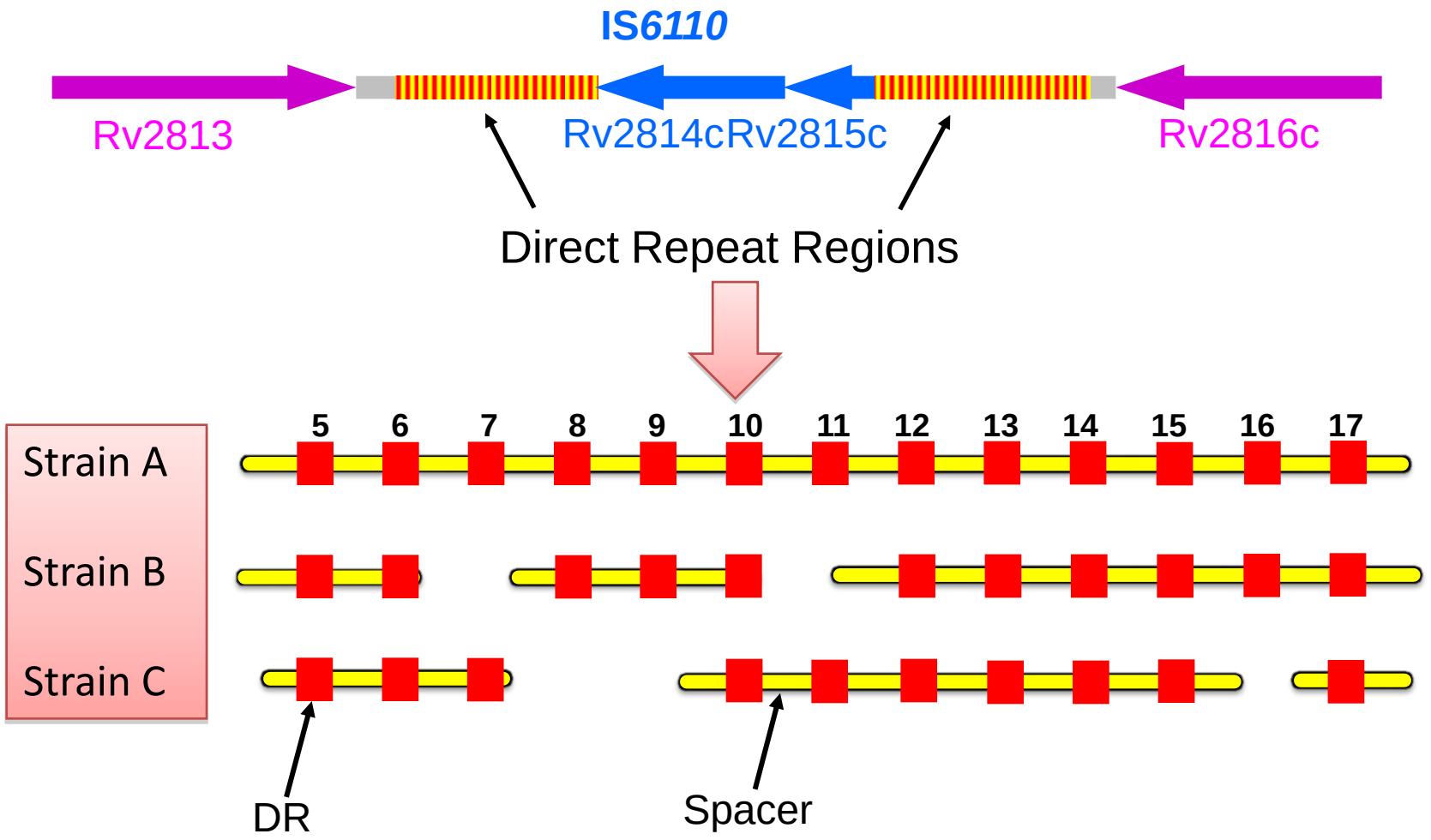
- **Pros**
  - Standardised protocols
  - No requirement for PCR
  - International database available
- **Cons**
  - Time consuming culture & DNA purification (5 days)
  - Complex analysis
  - Poor discrimination for low numbers of IS*6110*
    - *M. bovis*

(Van Soolingen (2001) *J. Int. Med*. **249**:1-26)

Spacer-oligo typing (Spoligotyping)

- Members of the *Mtb* complex carry one DNA segment named the Direct Repeat locus (DR)
- DR consists of multiple copies (10-50) of a 36 bp direct repeat
- Each repeat is separated from the next by non-repeated variable “spacer” DNA (37-41 bp)
    - The total number of spacers varies between strains
- Copy of IS*6110* is inserted in the DR in all *M. bovis* and most *M. tuberculosis* strains

Differences in DR among strains in the *Mtb*-complex



>100 spacers found, but only 43 are used for Spoligotyping

Overview of the methodology

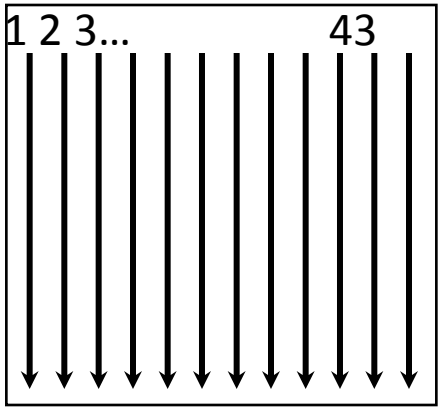
Link “spoligos” to the membrane

genomic DNA of strains to be typed

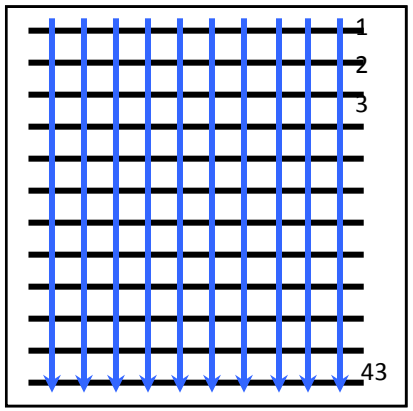
PCR amplify

label with Biotin

denature



Rotate membrane 90°

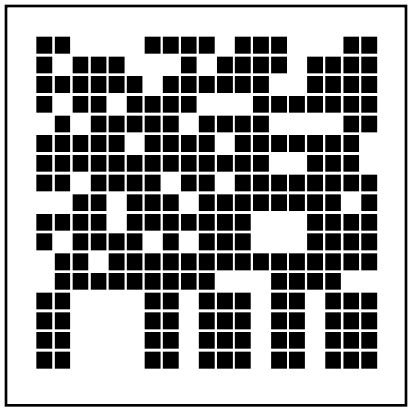


Hybridisation

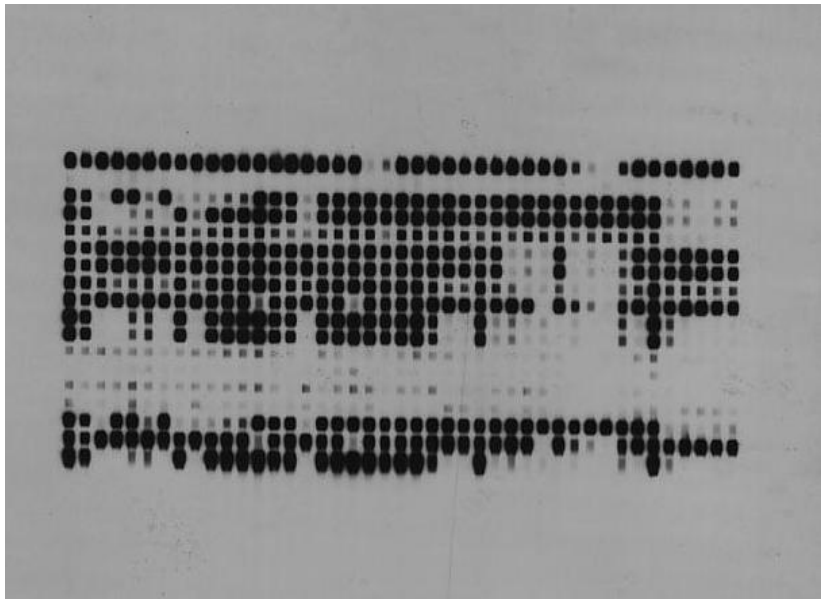
stringent wash

Strep-peroxidase

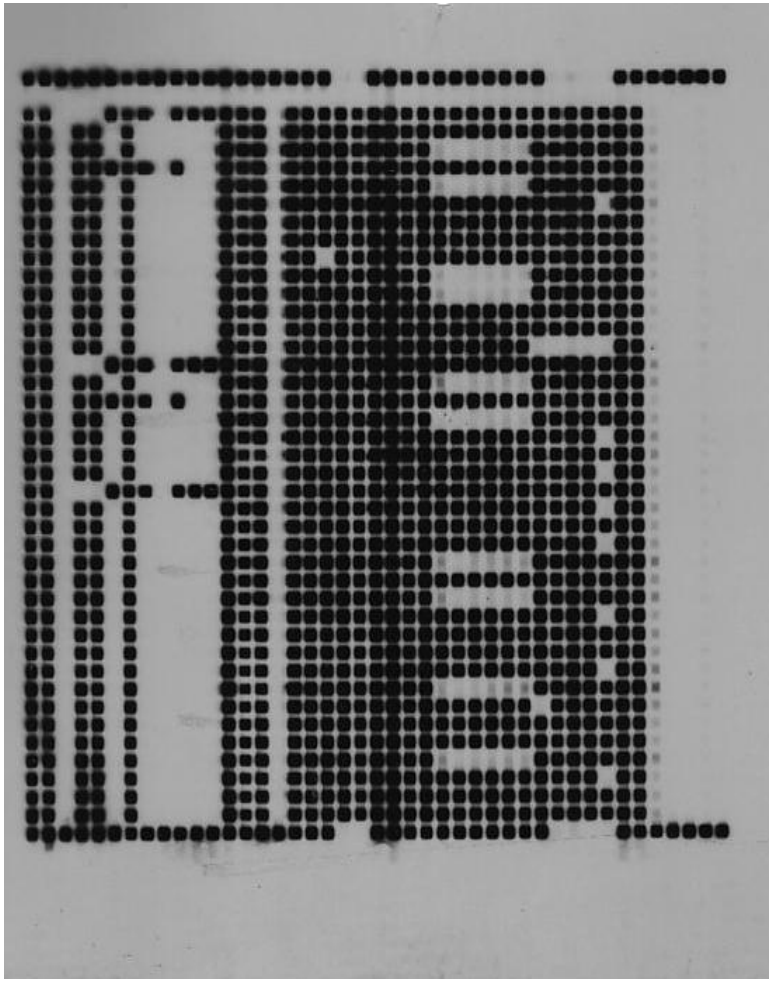
ECL-detection system/film



Examples of spoligotype results



1----------43 spacers



Banding pattern ⟶ 43 digit binary code ⟶ 15 digit octal code ⟶ analysis (automated)
(1= band present and 0= band absent)

Summary - Spoligotyping

- Spoligotyping uses the direct repeat region
- 43 spacer sequences were selected for this PCR and hybridisation-based technique
- Spoligotyping is suitable for typing of "IS*6110* low-copy-number" strains *e.g. M. bovis*
- Spoligotyping can discriminate *M. tuberculosis* from *M. bovis*

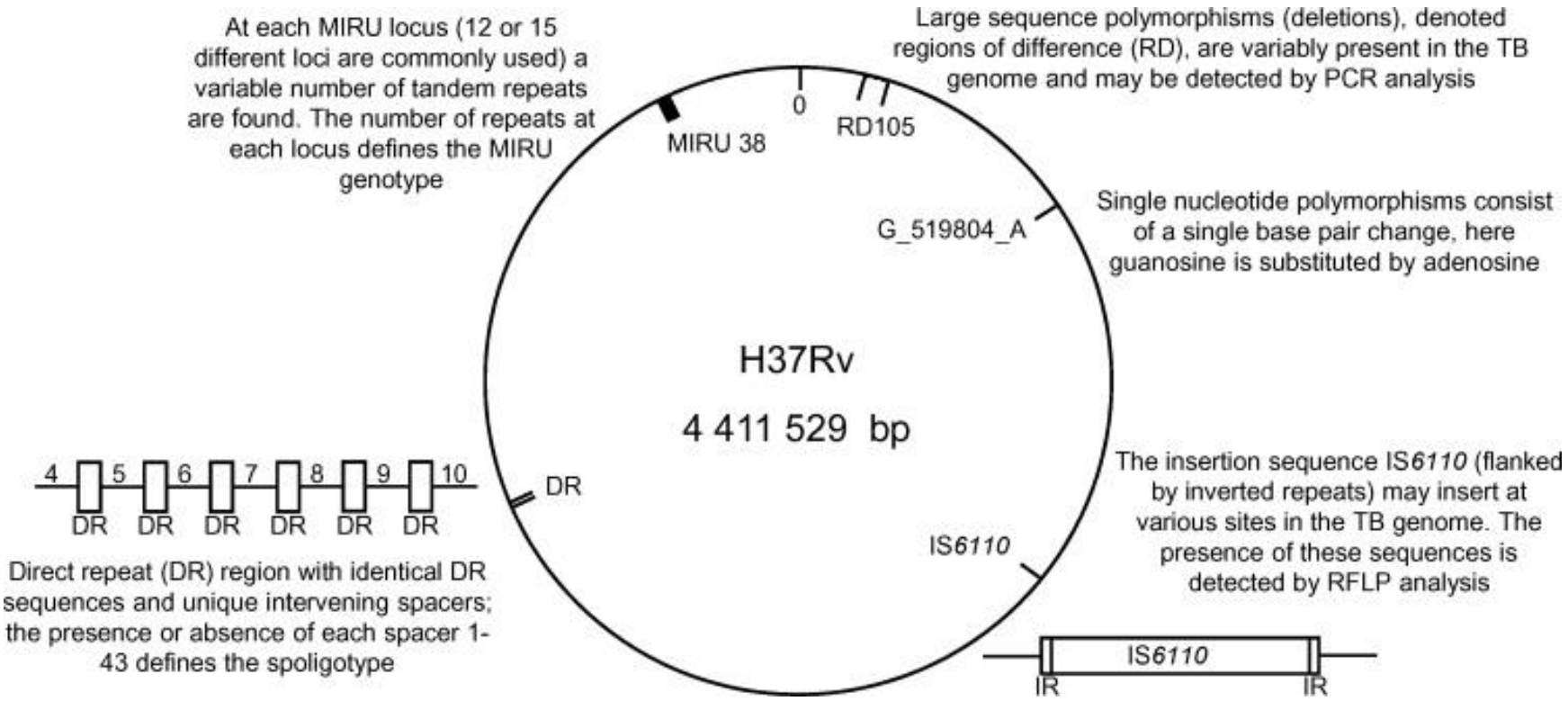
Large Sequence Polymorphisms (LSPs)

- Genetic diversity generated through large chromosomal deletions (RD regions)
- Comparative genome hybridization (DNA microarrays) to identify deletions
- Unique and irreversible
- Deletions used to define lineages
- Useful for identifying evolution of strains

Single Nucleotide Polymorphisms (SNPs)

- Phylogenetically informative mutations
- Conventionally, strains classified into genetic groups based on SNPs in *kat*G, *gyr*A and *rpo*B
- SNPs identified *in silico* by comparing multiple whole genome sequences

Summary-defining phylogenetic associations



Nicol M et al, Transactions of the Royal Society of Tropical Medicine and Hygiene.2008

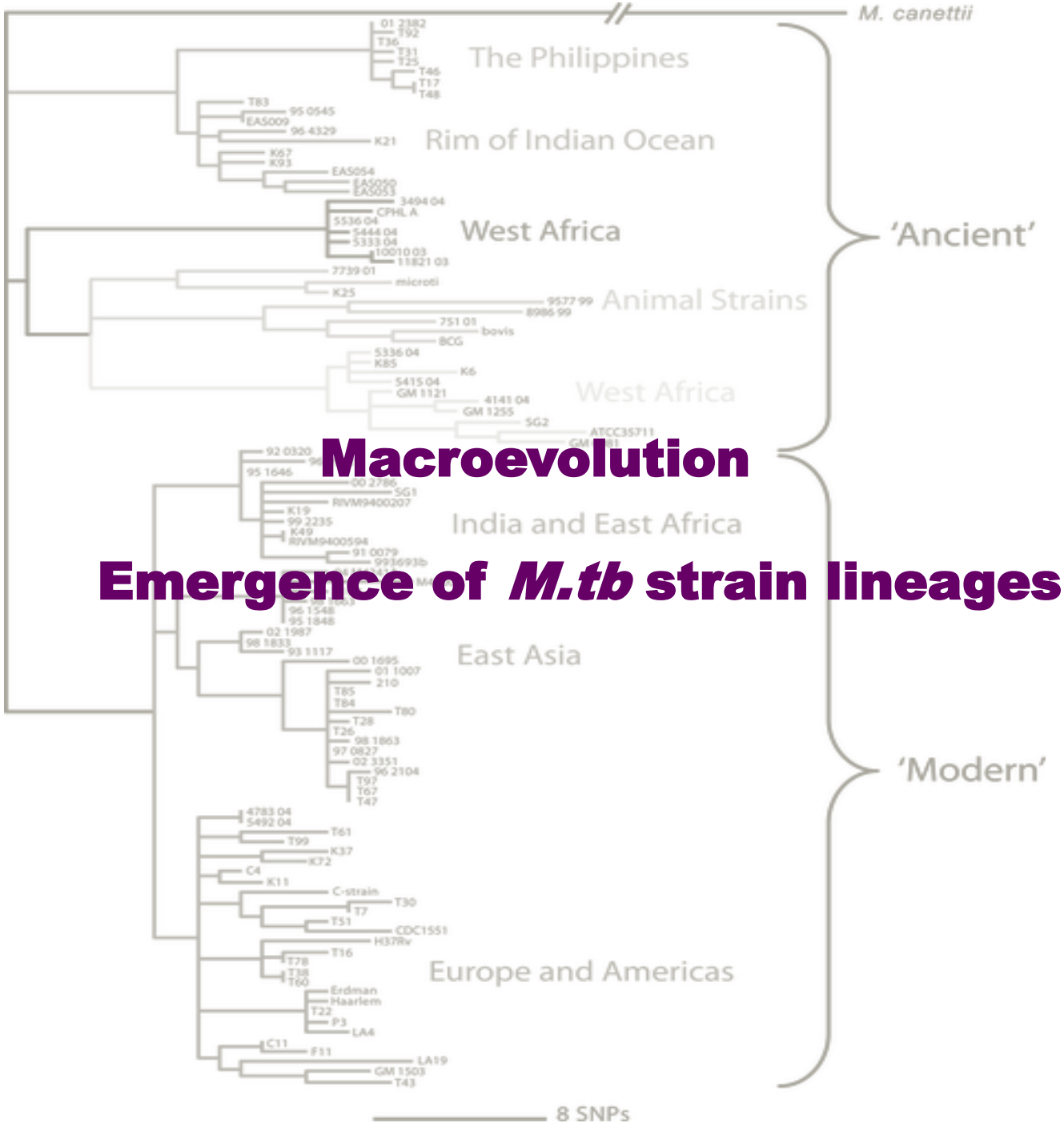
Macroevolution vs Microevolution

Macroevolution

- Evolution at the species level or higher
- Leads to fundamental differences between strain lineages

Microevolution

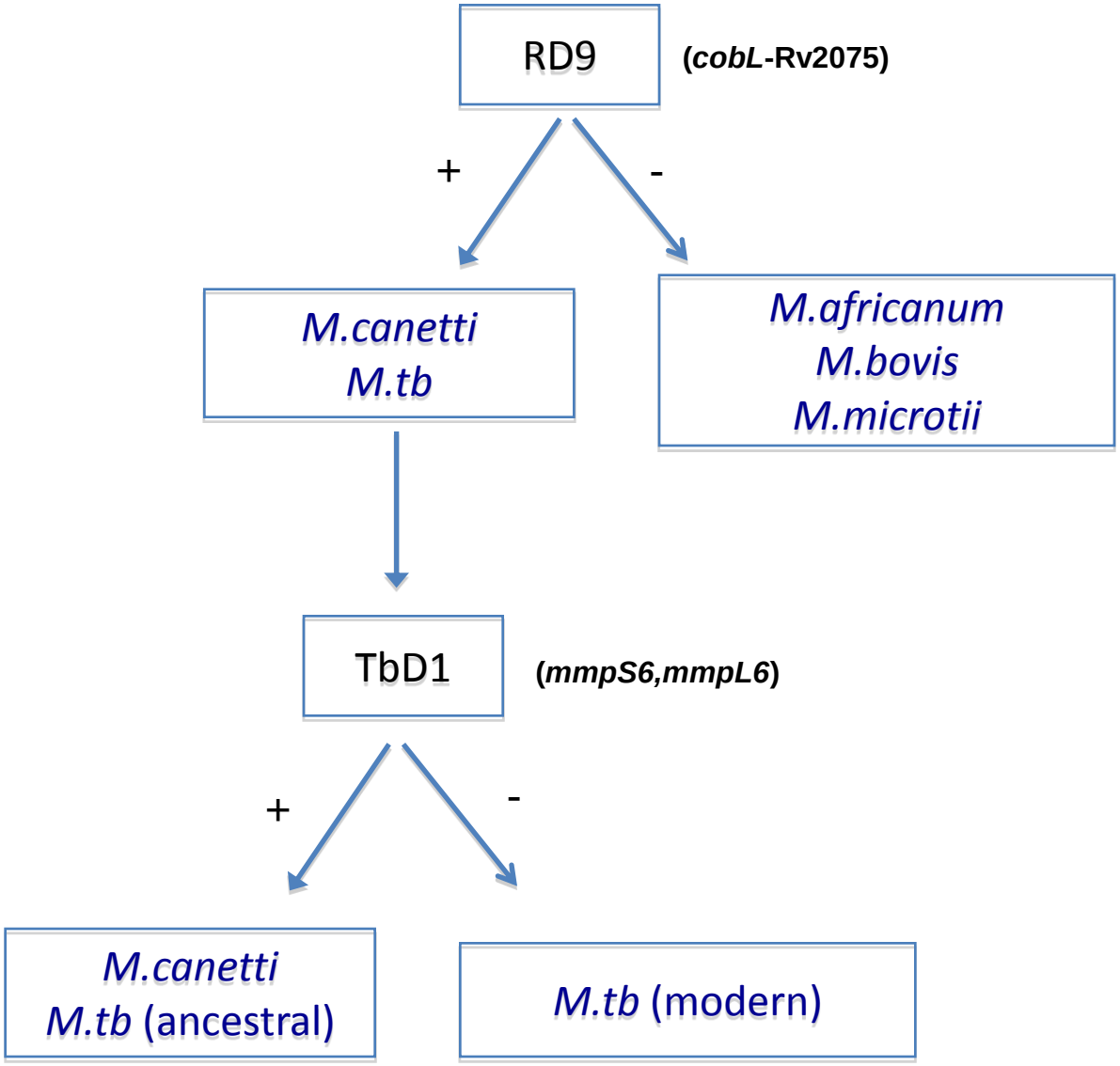
- Small evolutionary changes within a population
- Changes in allele frequencies
- Arises due to selection, mutation and genetic drift within a population
- Important role in the emergence of drug resistant mutants



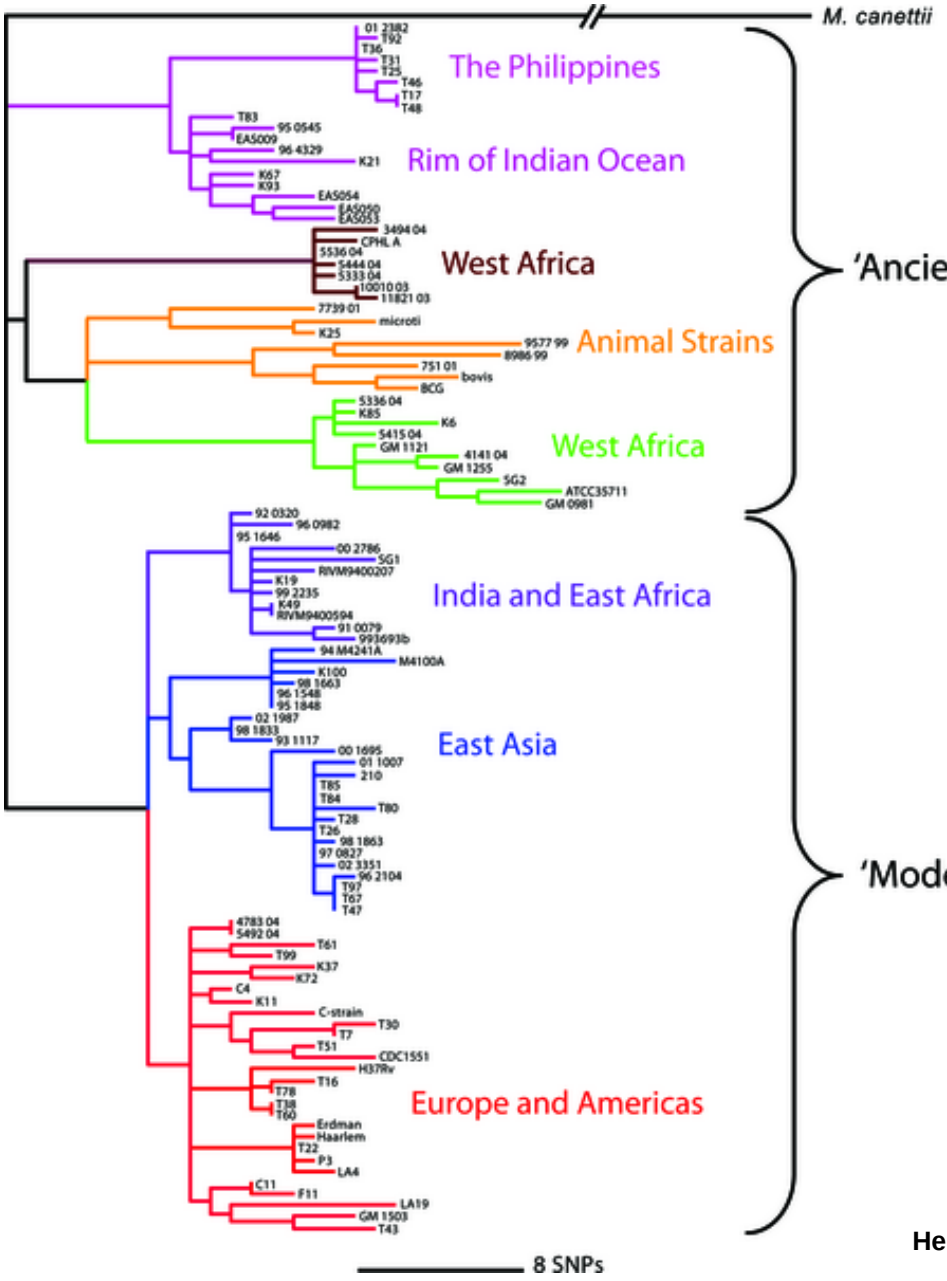
Macroevolution

Emergence of *M.tb* strain lineages

TbD1 deletion



Ancient vs Modern lineages of *M.tb* complex

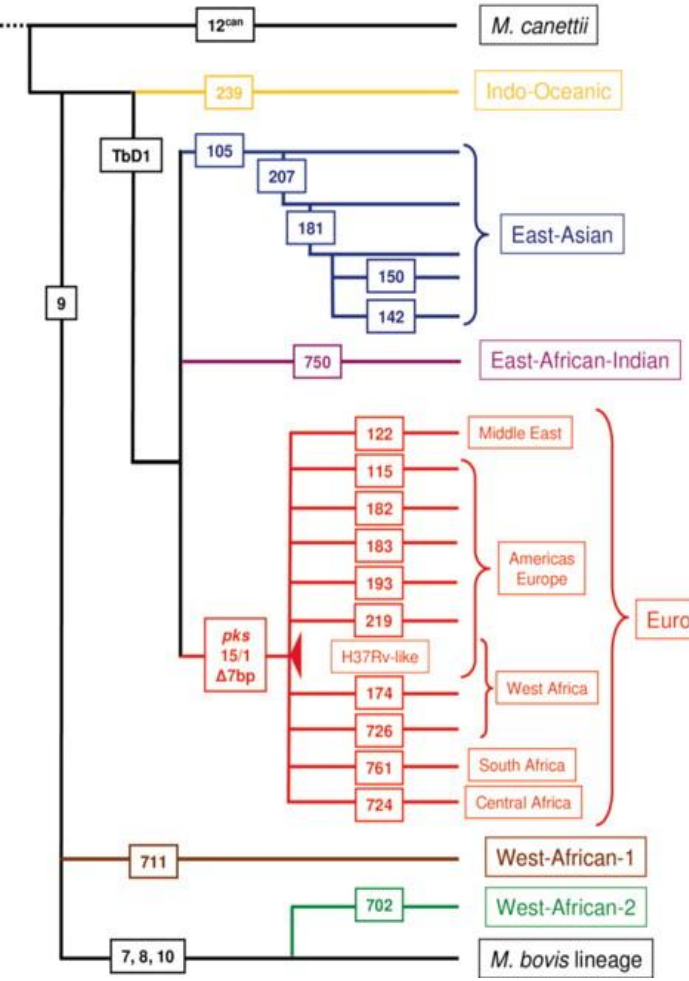


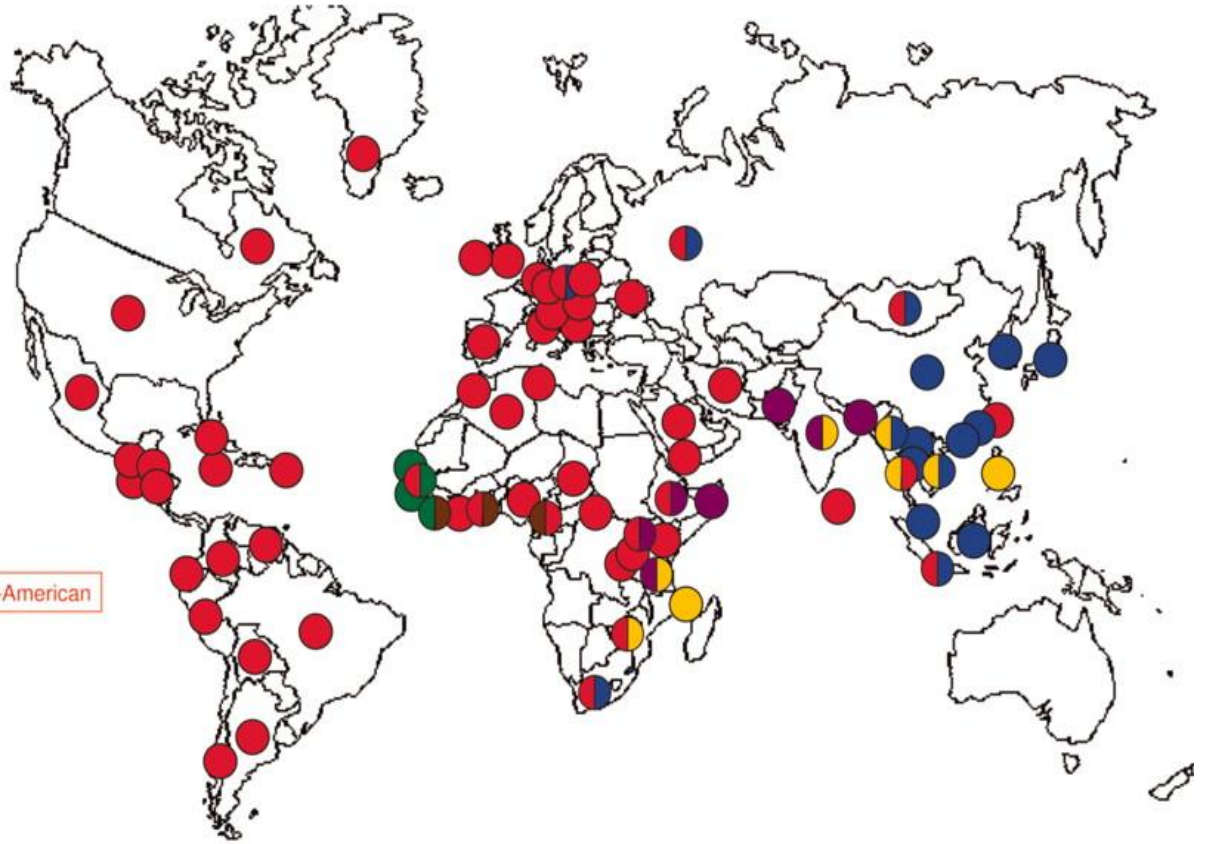
TbD1 +

TbD1 -

Hershberg et al,PLoS Biology.2008.

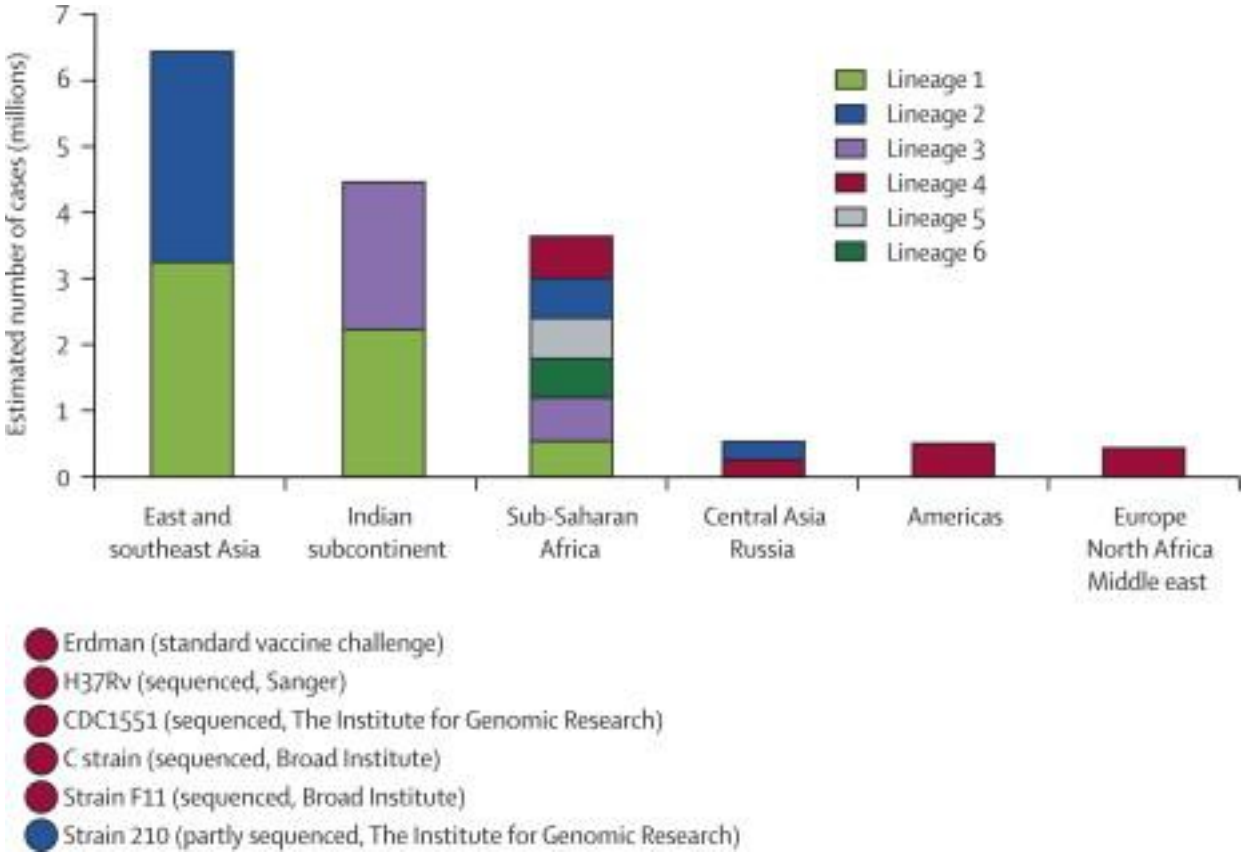
Geographical distribution of *M.tb*





Gagneux S et al. PNAS.2006

M.tb lineages- global TB burden (2002)



Gagneux S et al, Lancet Infect Dis. 2007.

Genetic markers

	Lineage 1	Lineage 2	Lineage 3	Lineage 4	Lineage 4	Lineage 5
SNP (Sreevatsan et al)	Principal genetic group 1	Principal genetic group 1	Principal genetic group 1	Principal genetic groups 2 and 3	Principal genetic group 1	Principal genetic group 1
SNP (Baker et al)	Lineage IV	Lineage I	Lineage III	Lineage II	Not done	Not done
LSP (Gagneux et al)	Indo-Oceanic lineage	East Asian lineage	East African-Indian lineage	Euro-American lineage	West African lineage I	West African lineage II
SNP (Gutacker et al)	Cluster I	Cluster II	Cluster II.A	Clusters III-VII	Not done	Not done
SNP (Filliol et al)	Cluster group 1	Cluster group 2	Cluster group 3a	Cluster groups 3b-6b	Not done	Not done
Spoligotyping (Brudey et al)	EAI	Beijing	CAS	Haarlem, LAM, T, X	AFRI2	AFRI1
LSP marker	RD239	RD105	RD750	Pks15/1 (7bp del)	RD711	RD702
SNP marker	OxyR C37T93	Rv3815c G81A68	RpoB T2646G	KatG T1388G, RpoB C3243T	Not known	Not known
Geographical association	East Africa,S.E.Asia, S.India	E.Asia, Russia, S.Africa	E.Africa, N.India, Pakistan	Americas, Europe,N.Africa	Ghana, Benin, Nigeria, Cameroon	Senegal,The Gambia
	Similar to ancestor (TbD1)				*M.africanum* subtype 1 (clade1)	*M.africanum* subtype 1 (clade2)

Gagneux S et al, Lancet Infect Dis. 2007.

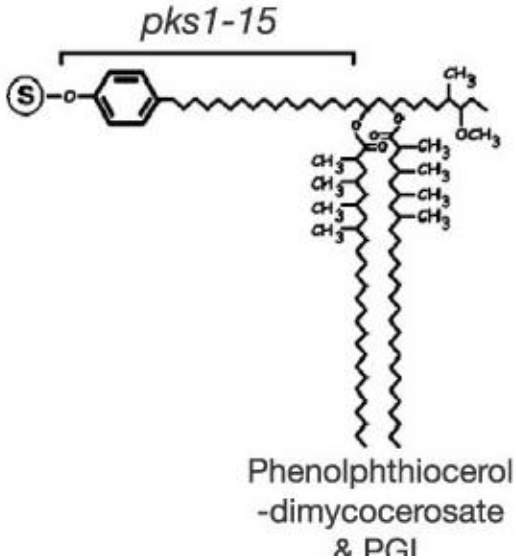
Phenotypic consequences of strain diversity

- Immune modulation
- Virulence
- Clinical outcomes

Outbreak strains-I

HN878 (W-Beijing strain)

- Outbreak of TB in Houston between 1995-1998
- Consistent hyper-virulent phenotype in infection models
- Increased dissemination, rapid death in mice and high bacterial burden in a rabbit model (Manca et al, 2000; Tsenova at al 2005)
- Associated with reduced expression of pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ and IL-12)
- Increased production of type I interferons
- Hypervirulence associated with phenolic glycolipid (PGL)

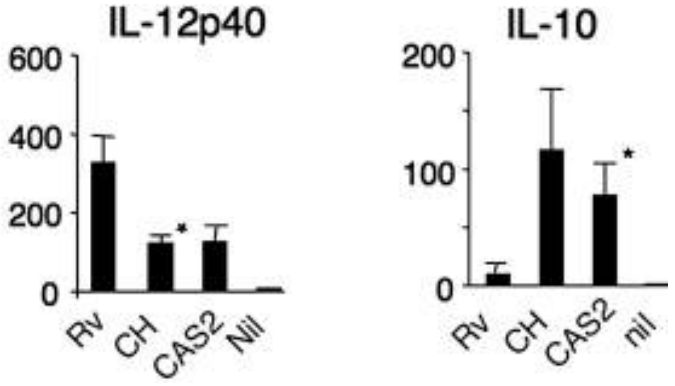


Reed et al, Nature.2004

Outbreak strains-II

Strain CH (East African-Indian lineage)

- Genomic deletion of region RD750
- About 23% of infected cases progressed to active disease in year 1
- *In vitro*, strain was associated with reduced IL-12p40 (protective) and increased IL-10 (anti-inflammatory/regulatory cytokine)

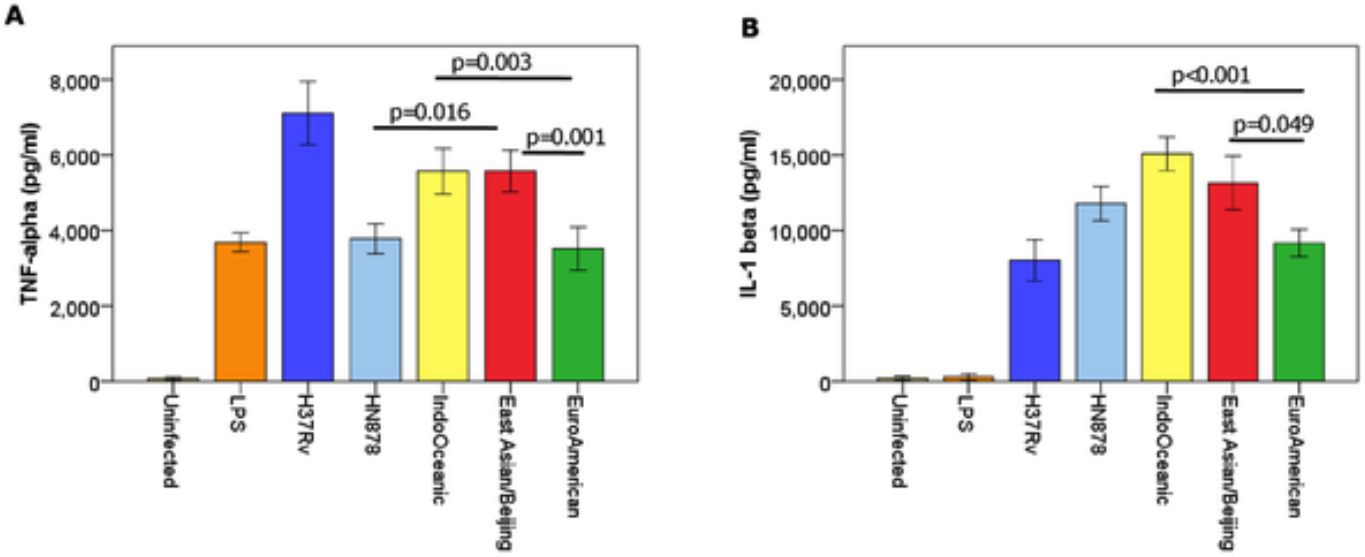


- Phenotype associated with inactivation of Rv1519

Newton et al, PNAS.2006

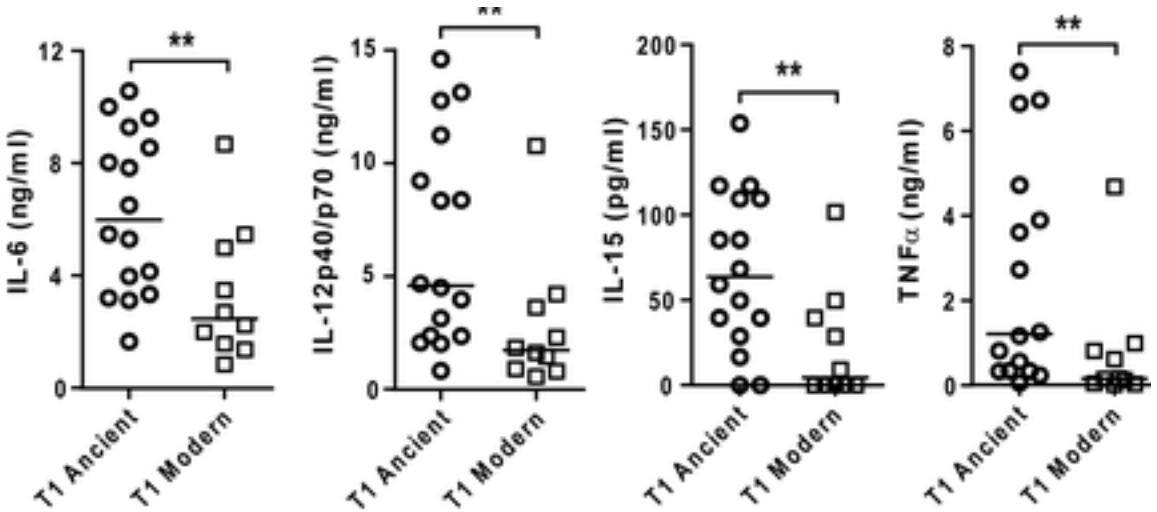
Strain dependent immune phenotypes

Mycobacterial lineage influences cytokine expression from infected mouse macrophages



Krishnan et al, PloS One. 2011

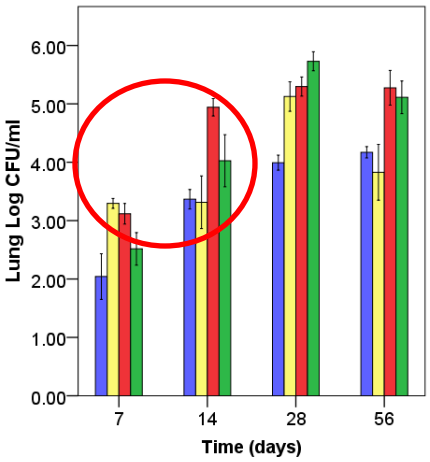
Strains from the modern lineages induce lower levels of pro-inflammatory cytokines

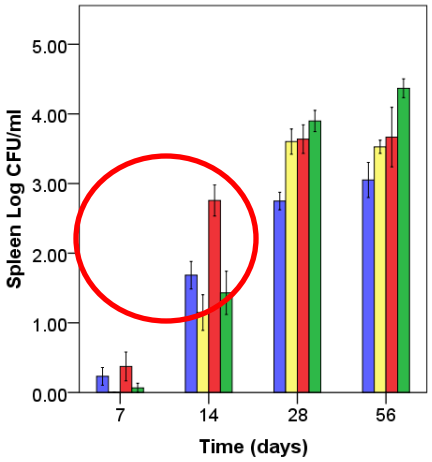


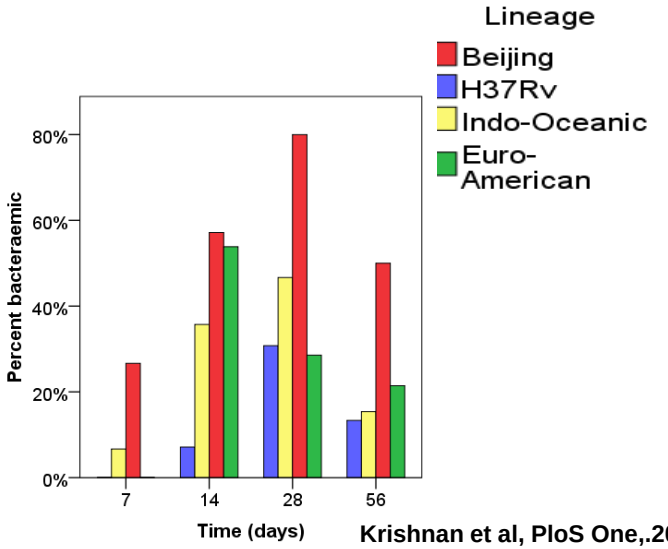
Portevin et al, PloS Pathogens. 2011

Lineage specific virulence patterns

- South Indian isolates of *M.tb* were attenuated in the guinea pig model (Mitchison et al, 1960)
  - Lower levels of sulpholipids
  - Highly susceptible to peroxide stress
- W-Beijing strains
  - More associated with extra-pulmonary TB (Kong et al, 2007)
  - Associated with treatment failure and relapse
  - Increased world-wide prevalence
- East Asian/Beijing lineage
  - Associated with increased bacterial load in the lungs and spleen (early time points)
  - Displays prolonged bacteraemia

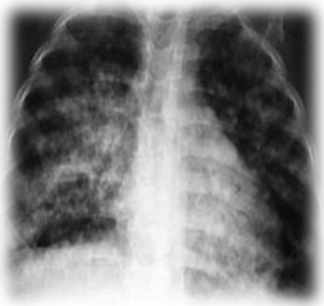




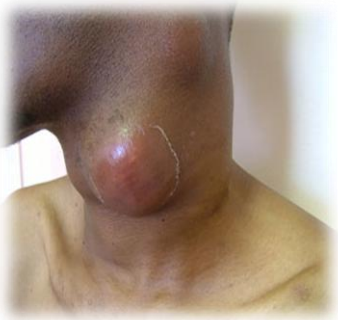


Krishnan et al, PloS One,.2011

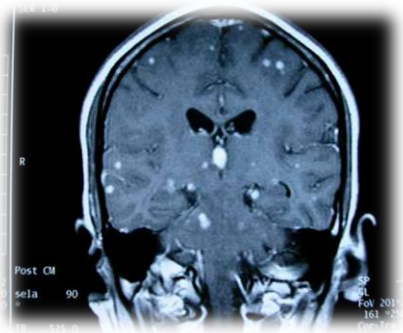
Clinical consequences of strain diversity



In Vietnam, Euro-American strains were more associated with pulmonary disease

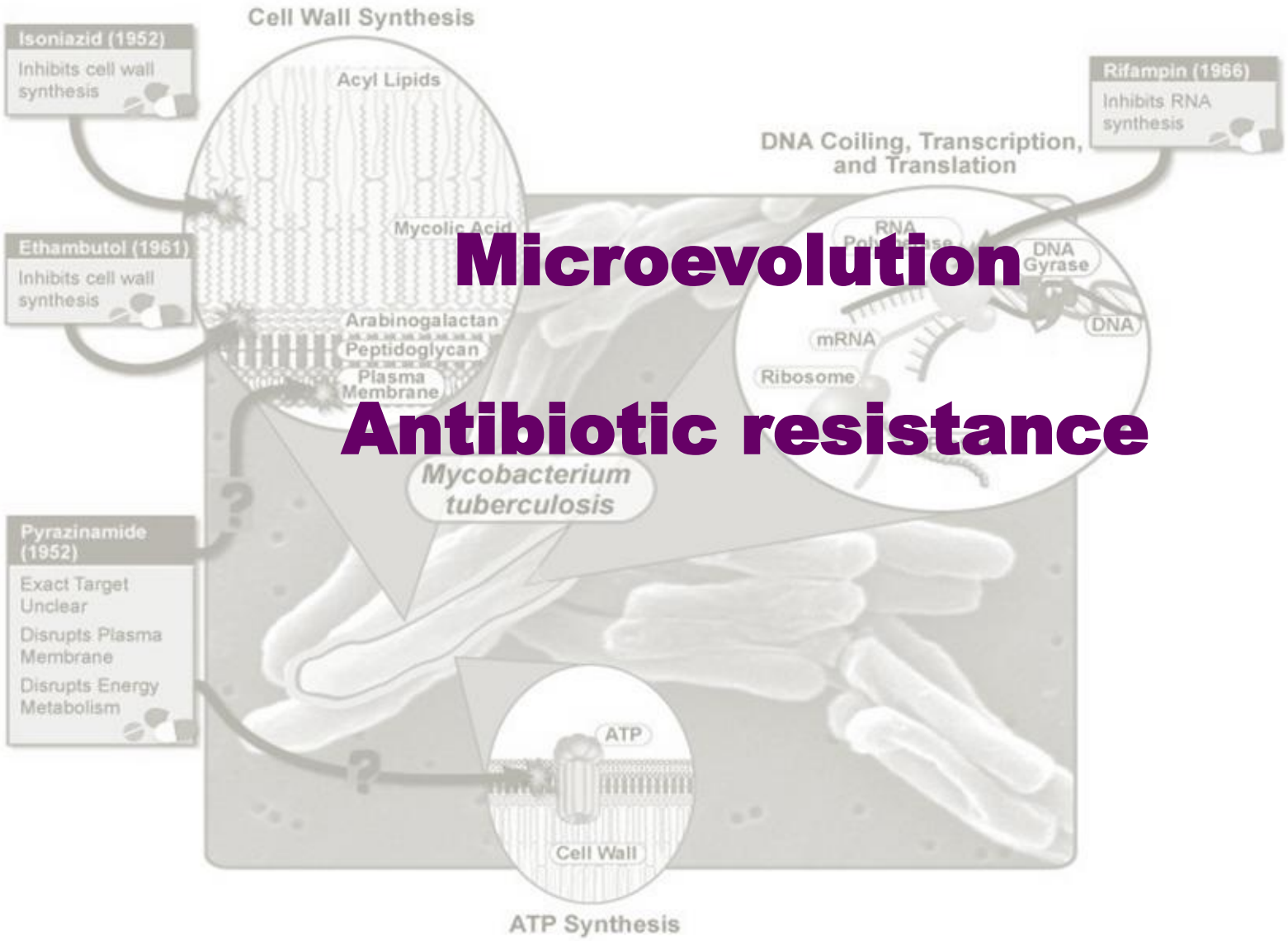


Association between CAS strains and extra-pulmonary disease



Beijing and Indo-Oceanic strains were more associated with TB meningitis

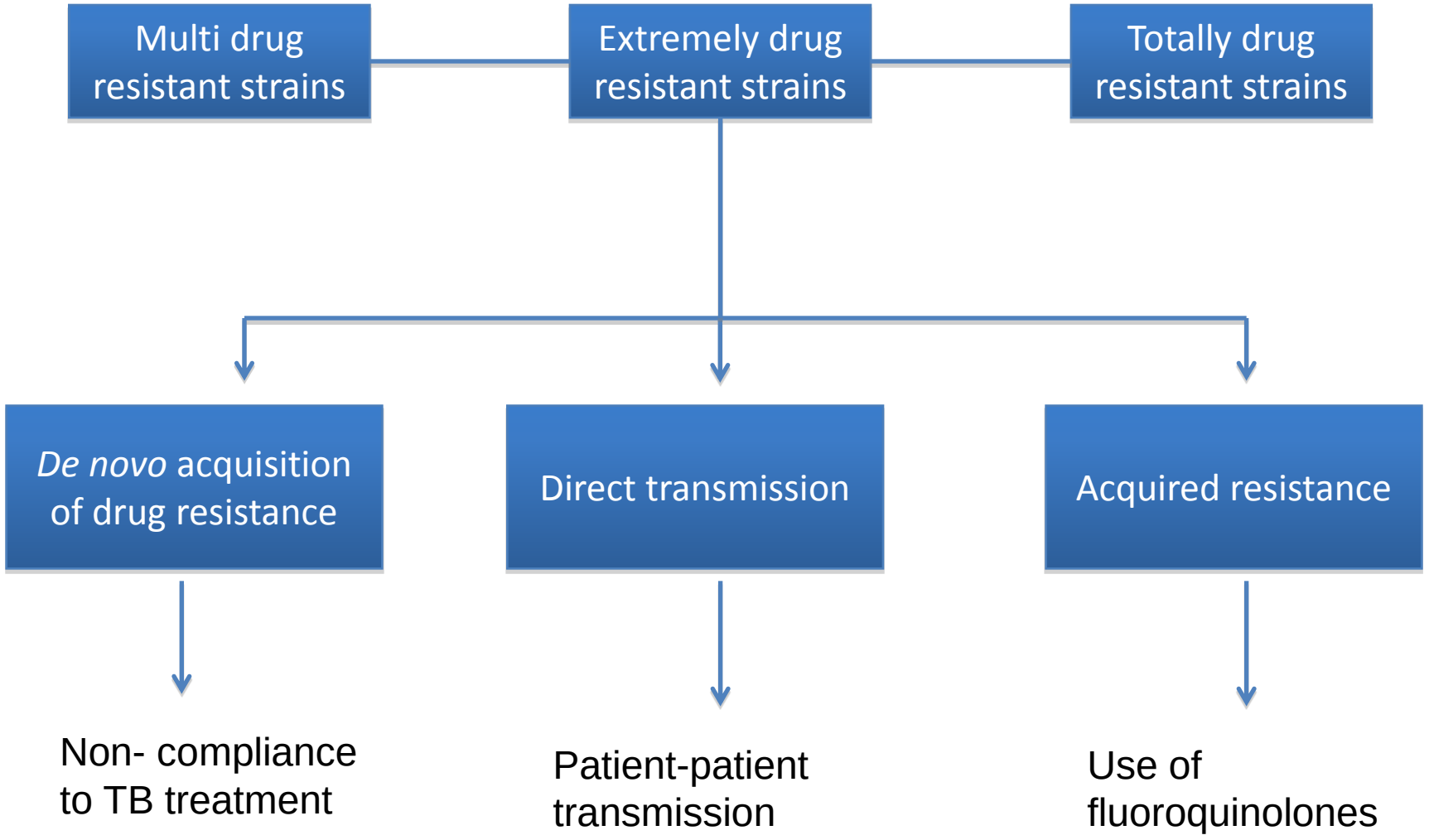
Thwaites G. et al, JCM.2008
Lari et al, 2009



Microevolution

Antibiotic resistance

Acquisition of drug resistance



Drug resistance

- Fitness factor

- Strong selection for low fitness cost mutations (clinical)
  - MDR-TB often associated with HIV
  - Compensatory mutations can overcome fitness defects

- Strain genetic background can affect resistance mutations
  - Beijing family frequently associated with drug resistance

Implications for drug and vaccine development

- Certain lineages can harbour mutations (SNPs) that would make them naturally resistant to drugs
  - *M.bovis* and resistance to pyrazinamide
  - nsSNP in *pncA*

- Predominance of Beijing strains in areas with widespread coverage of BCG

- *M.tb* antigens maybe differentially present in clinical isolates
  - Example: Mtb72F subunit vaccine composed of *M.tb* proteins ,PepA and PPE18
  - Vaccines may not provide uniform protection

- Targeting only a proportion of a specific population exerts selective pressure

Conclusions

- Molecular markers (e.g. LSPs and SNPs) aid in the classification of *M.tb* strains
- Lineages of *M.tb* are associated with particular geographical regions
- *M.tb* strains differ in immunogenicity and virulence (interplay between host and pathogen factors)
- The impact of strain variation on human TB disease is unclear
- Genetic background of strains can influence the evolution of drug resistance and determine effectiveness of new vaccines

Learning objectives

- Outline the role of molecular typing methods in defining phylogenetic associations

- Compare and contrast macroevolution and microevolution

- Describe the phenotypic consequences of strain diversity