Application of DNA-based methods to epidemiology of TB

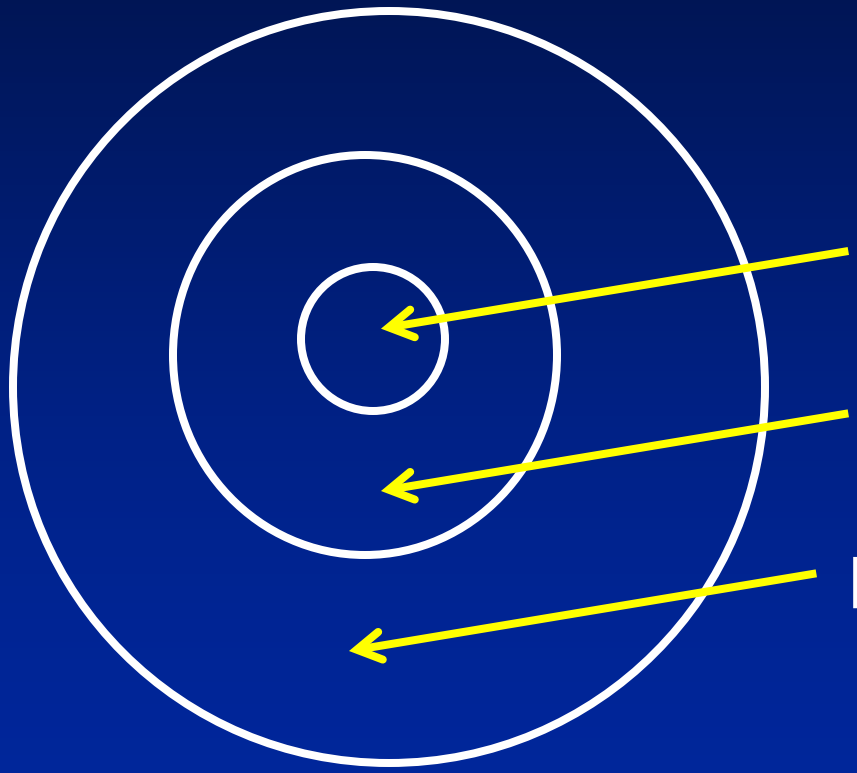
Marcel A. Behr

Professor, McGill University

Director, McGill Int. TB Centre

marcel.behr@mcgill.ca

Planes of molepi study



Individual = Clinician

Defined outbreak = Disease Control

Population = Epidemiologist

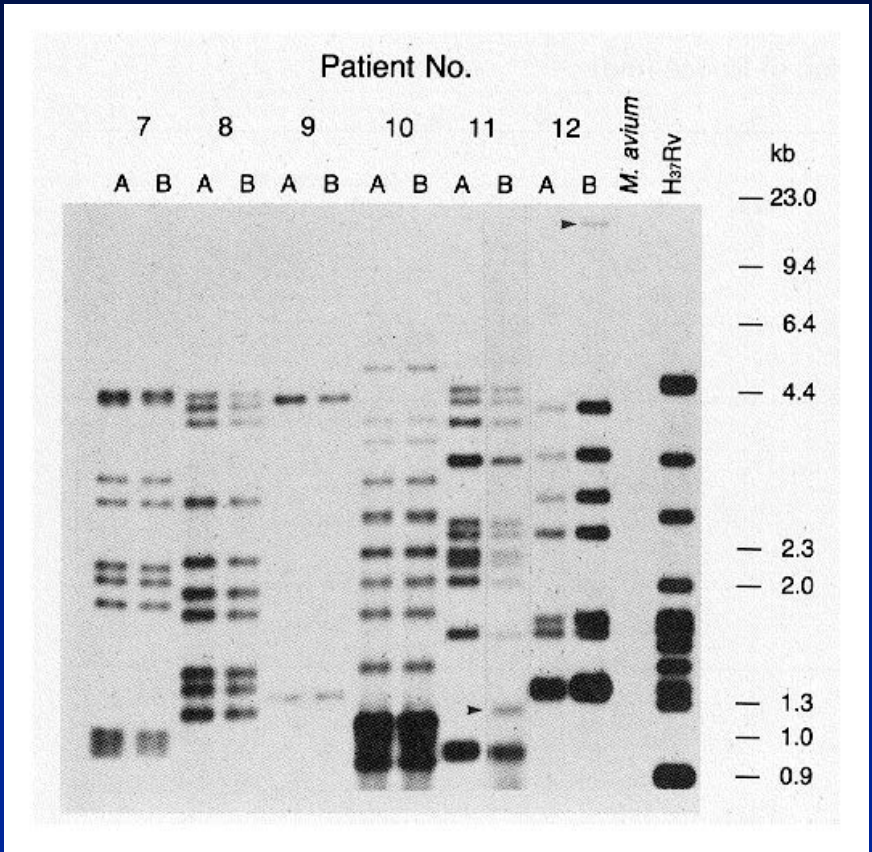
Some questions addressed by genotyping methods

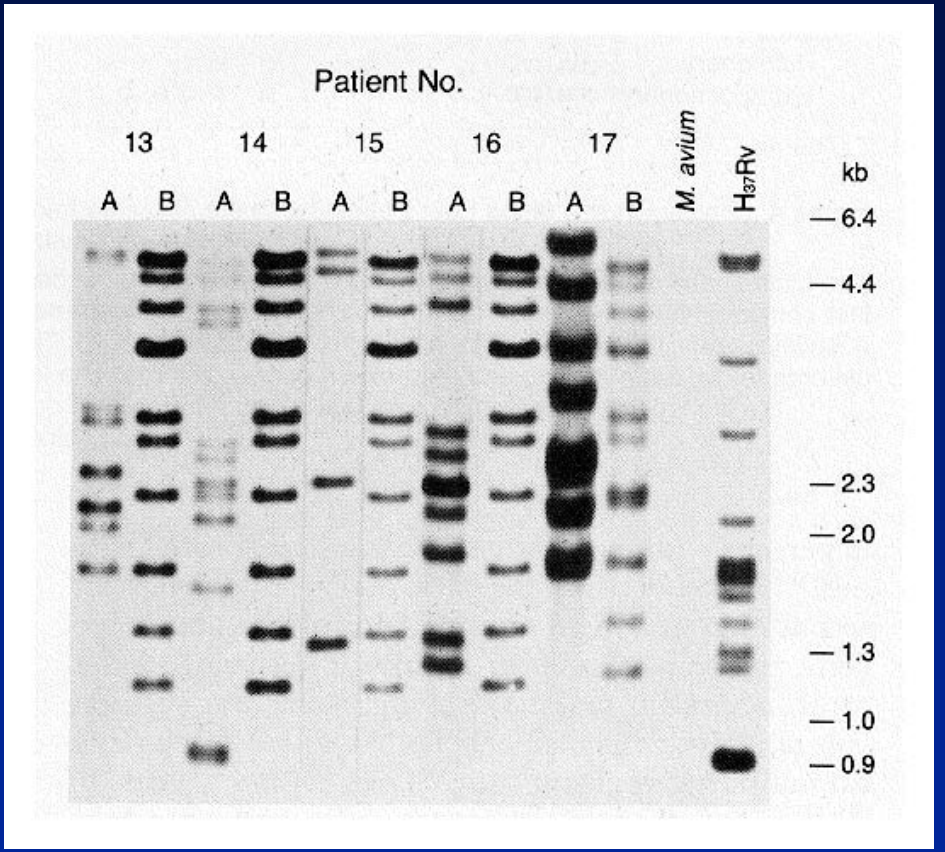
- Clinical:
  - Reasons for treatment failure?
- Immunology:
  - Are TB patients protected from TB?
- Epidemiology:
  - TB due to recent transmission?
- Bacteriology:
  - Do all strains behave equally?
- History:
  - How did TB spread around the globe?

Clinical: Molepi of recurrence

- TB, Rx then TB again
  - Is it relapse? Clinic problem
  - Is it reinfection? Public health problem
- Change in antibiotic resistance
  - Could be acquired drug-resistance
- No change in antibiotic resistance
  - Could be a new strain
- Antibiotic phenotype unreliable to judge relapse vs. reinfection

RFLP of DS to MDR-TB





Relapses have original strain

Reinfections - ‘house’ strain

Small et al., NEJM, 1993

Classification of recurrence

- Compare initial to recurrent isolate
  - Match = Relapse
  - Different = Reinfection
- South Africa:
  - 75% of those with recurrent TB after treatment have reinfection (new strain)

    Van Rie, NEJM, 1999
  - Cases classified by WHO as acquired drug resistance were reinfection

    Van Rie, Lancet, 2000

Relapse vs reinfection

- Distinction critical in RCTs
  - Reinfection cases would otherwise decrease estimated efficacy of therapy
- Standard now is to include first and recurrent isolate in studies
- Most recently done using Whole Genome Sequencing
  - Relapse vs. Reinfection vs. Mixed infection

Bryan, Lancet Resp Med, 2013

Immunology of recurrent TB

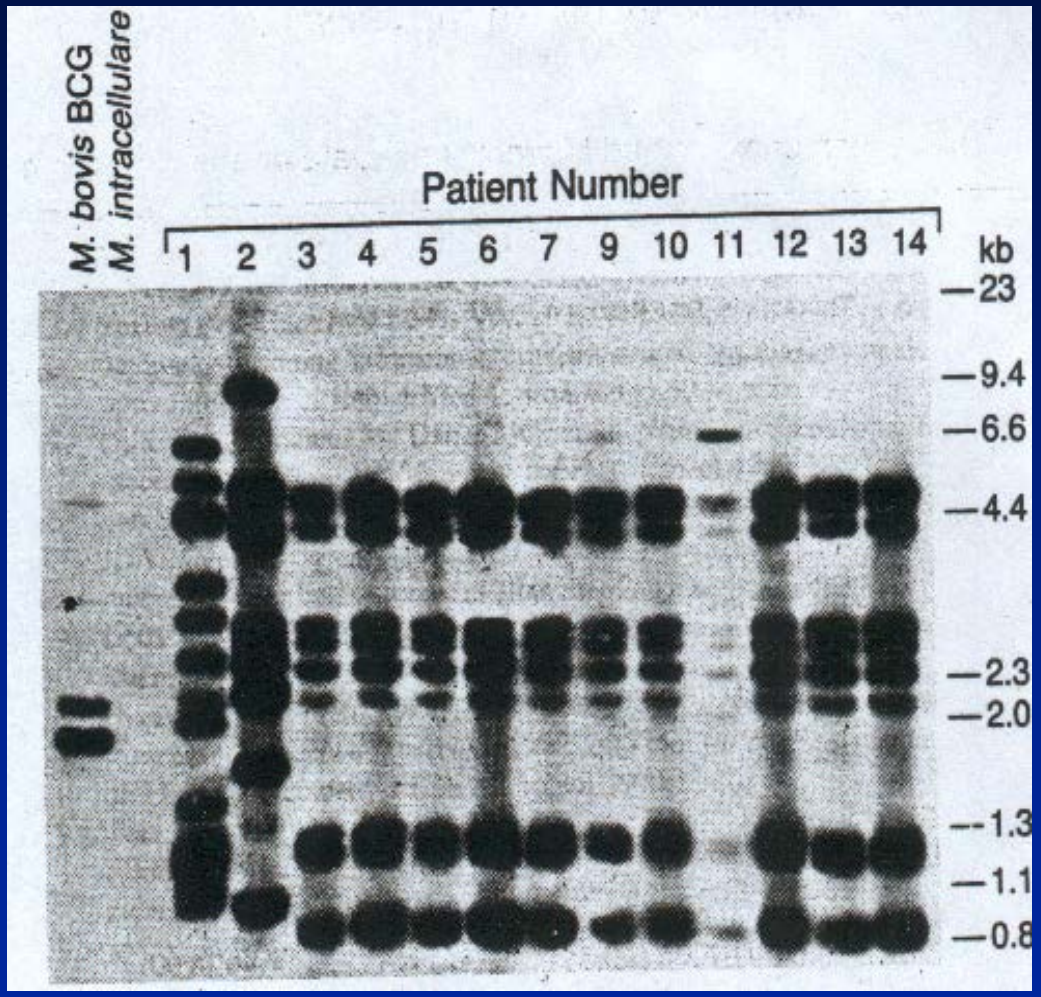
- People with prior positive TST have lower rate of TB
  - TB infection protects against new TB
  - TB infection is a marker of a survivor
- Does treated TB disease confer protection against new TB?
  - Practical importance
    - TB contacts previously treated for TB?
  - Immunologic value
    - Can we make a vaccine?

Immunology: TB again

- To determine risk of new TB, need to distinguish relapse from reinfection
  - Exclude treatment failure; new infection only
- Capetown study
  - Previously treated with new RFLP
  - 5x rate of TB compared to community
    - Suggests that those who could not control bacteria first time cannot control it the next time

      Verver, Am J Resp CCM, 2004
  - I am unaware of any other study that has looked at this….yet

Epidemiology: Outbreaks



- Case 1 & 2 unrelated
- 3 started outbreak
- 12 cases in 100 days
- Min. incubation period

< 4 weeks

From Daley et al., NEJM 1992

Outbreaks in a population

- Outbreak isolates share genotypes
- Therefore: If all isolates in city typed, those with same genotype are ‘outbreaks’
- Called clusters:
  - Percent cases in community clustered a proxy for ongoing transmission
  - Risk factors for clustering used to guide interventions

Small et al, NEJM, 1994

Alland et al, NEJM, 1994

Sampling matters

- Clustering studied in epidemiologically-defined space and time
  - Years better than months
  - Island is ideal
- ‘Edge effects’ reduce clustering
- Undersampling reduces clustering
  - 1000 people: 449500 pairwise tests
    - 800 isolates: 63% of pairs tested
    - 600 isolates: 36% of pairs tested
  - Risk of bias, depending on source of isolates

Studies of TB clustering

- Outcome measured:
  - Typically proportion/percent TB clustered
  - Occasionally incidence of clustered TB
- Who is in clusters?
  - Typically test risk factors
    - E.g. HIV, homeless
  - Occasionally ask targeted question
    - E.g. smear-negative cases (Behr, 1999)

Clustering varies

- Over place
  - San Francisco ~ 40%
  - Montreal ~ 10%
  - Capetown ~ 70%
- Over time
  - San Franciso:
    - Unique cases unchanged over time
    - Clustered cases dropped with enhanced TB control

Jasmer, Annals of Int Med, 1999

Risk factors for clustering vary

- Is HIV a risk factor for clustering?
- Prevalent HIV/AIDS with new TB case
  - Outbreak of recently transmitted TB
- Endemic TB with new HIV
  - HIV drives reactivation disease
- HIV is risk factor for
  - Transmission
  - Reactivation
  - Ratio of these two may go up or down

Bacteriology: Are there a more or less successful strains?

- Many reports of clinical/epidemiology observation associated with strain x
  - E.g. Beijing strain and drug resitance
  - E.g. CDC1551 strain and high % TST conversion among contacts
- Is one *M. tb.* strain more likely to develop drug-resistance?
- Is there a more virulent strain?

Bacteriology: Phenotypes

- Drug-resistance
  - In theory straightforward
  - In practice not consistent worldwide
- ‘Virulence’
  - If a strain kills mice faster, does this predict:
    - More transmissible?
    - Less transmissible?
    - Ideal scenario for TB transmission: keep host alive with chronic, transmissible disease

Bacteriology: Genotypes

- RFLP/MIRU/Spoligotype unreliable
- Deletions or SNPs best suited to ‘brand’ strains in a study

- In molepi studies, local-born generally associated with transmission
- Thus, local strains often look more transmissible – people vs. bacteria?

Bacteriology: Genotypes

- Many reports of strains associated with resistance or transmission
  - E.g. Beijing and DR-TB in Russia
- Many other reports where no association
  - E.g. Beijing and anything in Montreal

Albanna, Plos One, 2011

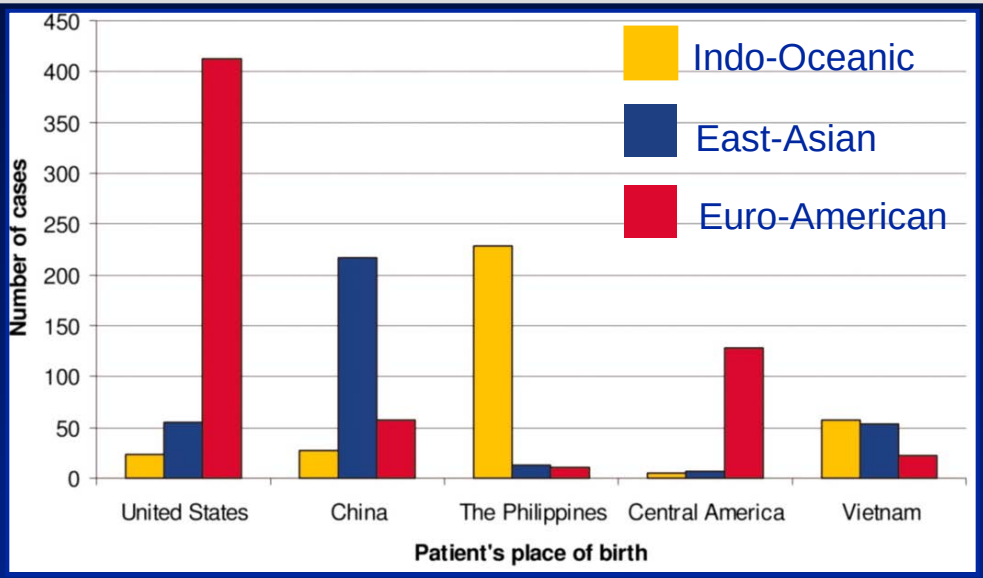
- Filter:
  - All isolates we study have most recently caused TB disease in a human
  - We don't get to study bacteria that fail to infect or fail to progress to disease

Using deletions to track *M. tb.* strains from around the world

- In San Francisco, 50 unique strains and 50 clustered strains
  - Tested by Genechip to look for deletions
- Patterns emerge:
  - Countries generally have a dominant strain
  - Strains can be seen across many countries

Hirsh et al, PNAS, 2004

Geography and strains: SF

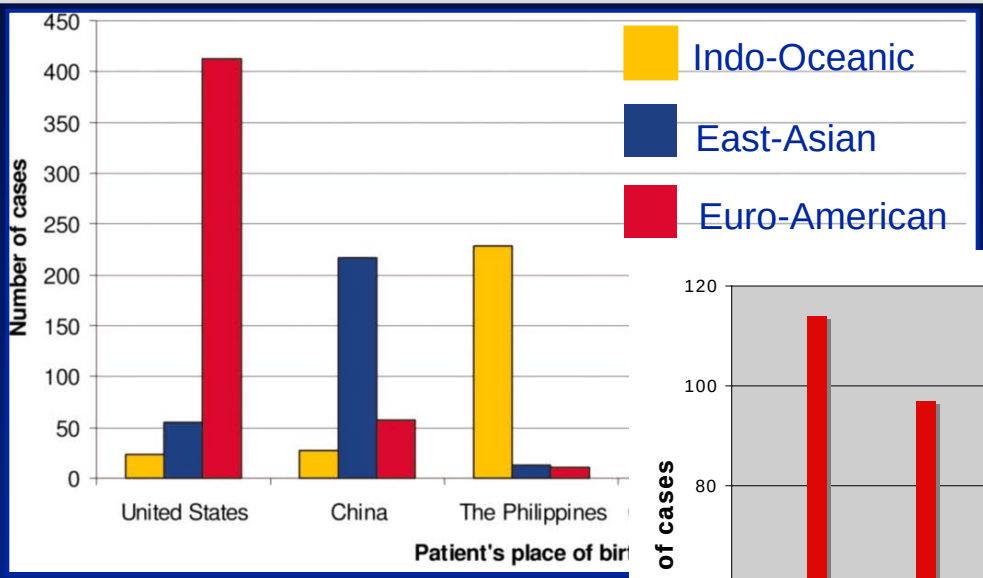


San Francisco

71% of TB cases
- 5 countries

Gagneux et al, PNAS, 2006

Geography and strains: Montreal



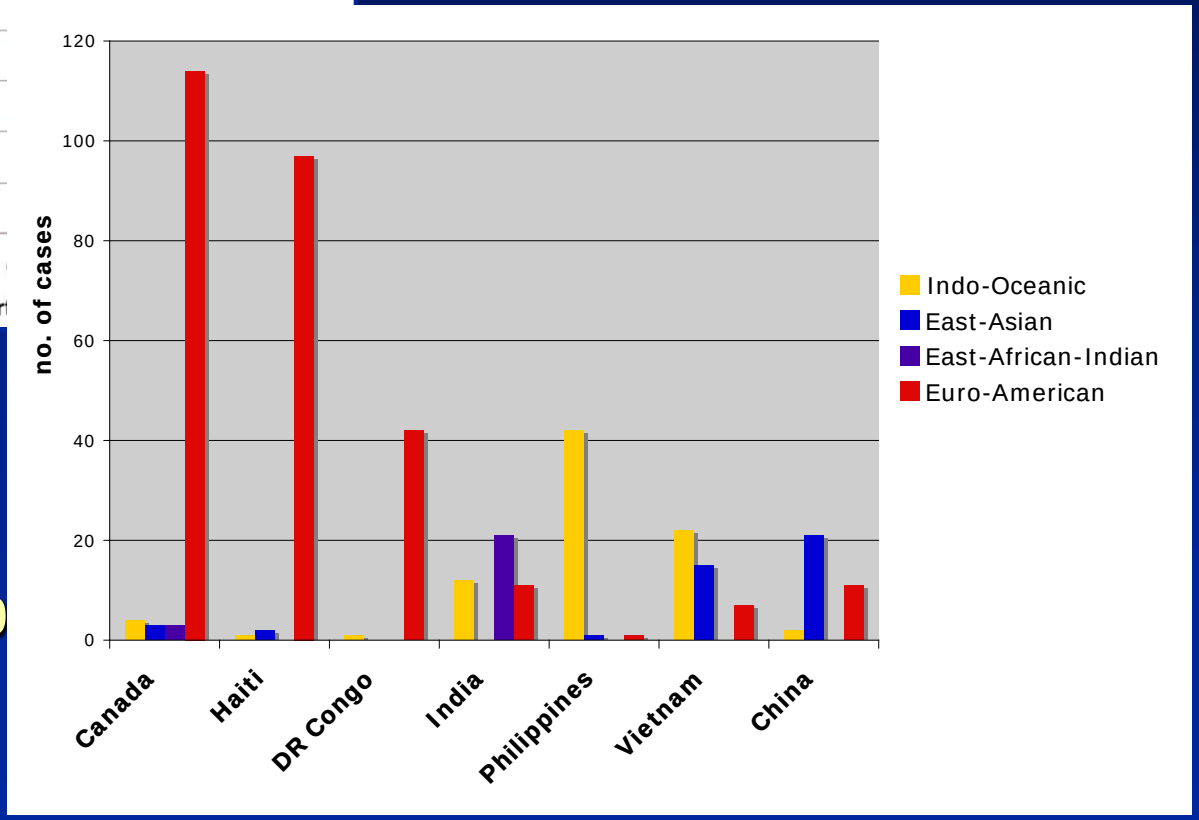
Montreal

60% of TB cases
- 7 countries

San Francisco

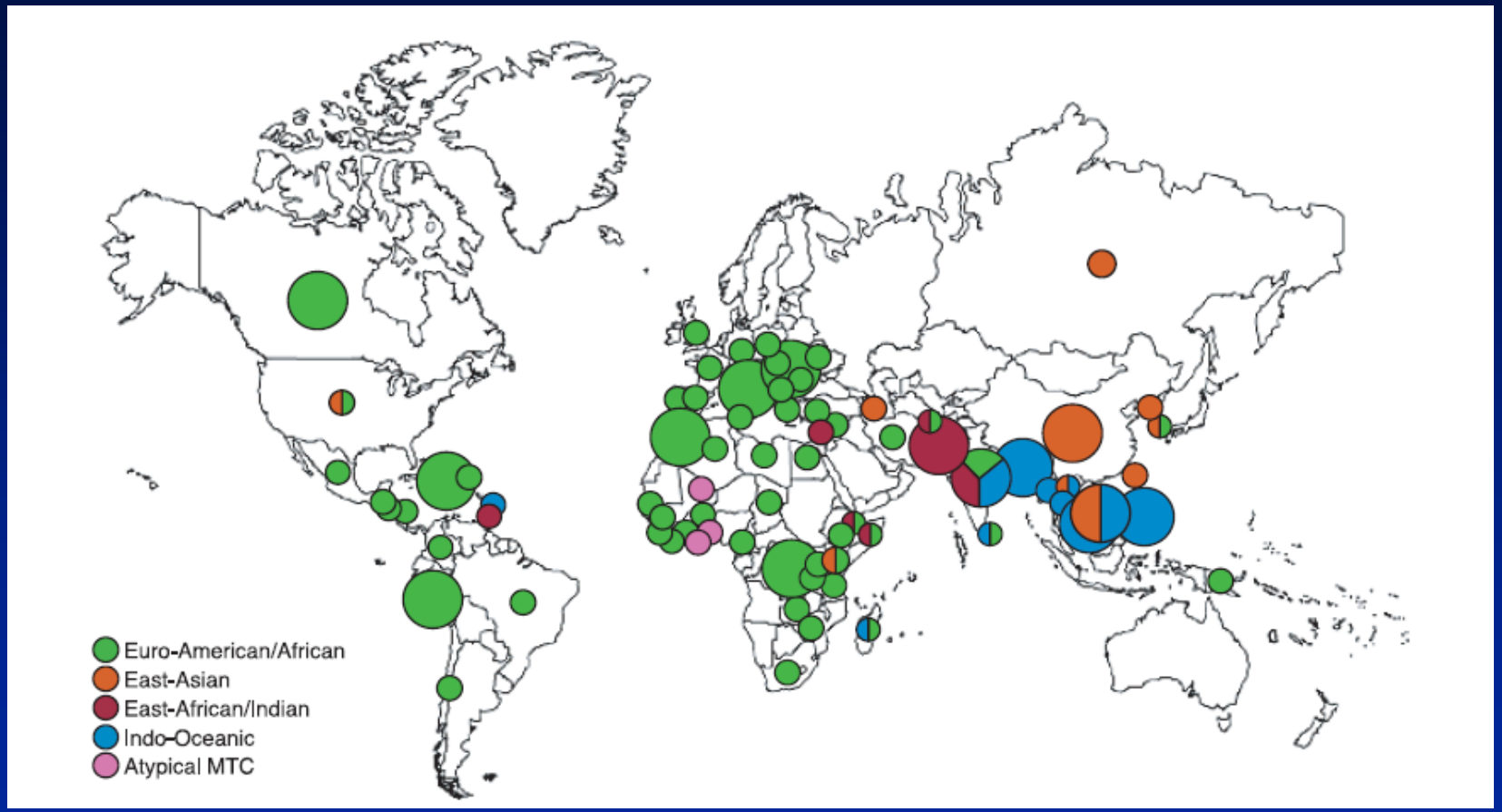
71% of TB cases
- 5 countries

Gagneux et al, PNAS, 20



Reed M et al, J. Clin Micro, 2009

M.tb strains & place of birth: Montreal



Reed M et al, J. Clin Micro, 2009

M. tb. spread through the ages

- ***M. tuberculosis*** **from Africa (all major lineages present)**
- ***M. tuberculosis*** **‘walked’ out of Africa with the paleo-migration**
- ***M. tuberculosis*** **then ‘sailed’ out of Europe during colonization of Americas**
- ***M. tuberculosis*** **‘canoed’ across Canada during the Fur Trade**

M. tb.: pathogen and symbiont

- ***M. tuberculosis*** **is a pathogen**
  - **Biomedical construct: causes disease**
- ***M. tuberculosis*** **is a symbiont**
  - **Biological construct: symbiosis is divergent organisms that live together**

Veyrier et al, Trends in Micro, 2011

M. tb.: pathogen and symbiont

- ***M. tb.*** **has been with us a very long time**
  - **Precarious balance**
- **When conditions favorable, TB rates go up**
  - **Countries with ↑ life expectancy have ↓ TB rates (early 20th century)**
  - **Countries with ↓ life expectancy have ↑ TB rates (late 20th century)**

Oxlade, IJTLD, 2009

Lessons from TB about molepi

- The rate-limiting step in molecular epidemiology is…..the epidemiology
  - Need patient data, epidemiologic data, historical data to interpret
- Typing method used must be tailored to the question being asked
  - Hard to use rapidly evolving typing tools to study historical phenomena
  - Impossible to use branding tools that define lineages to track outbreaks of transmission

Questions?

Centre international de TB McGill

McGill International TB Centre