TB in Canada:
From Sea to Sea to Sea

Marcel A. Behr
Professor, McGill University
Director, McGill Int. TB Centre
marcel.behr@mcgill.ca

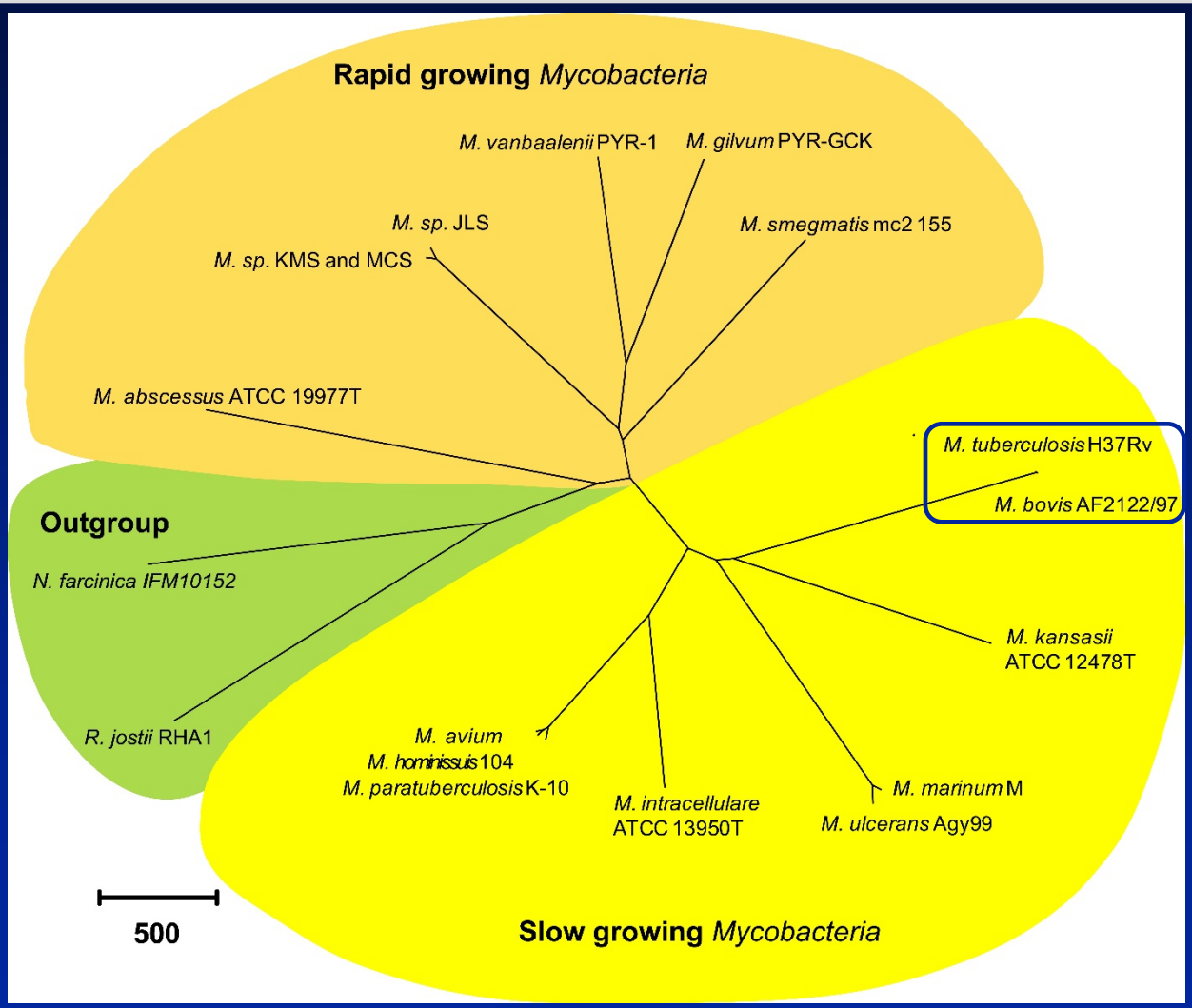
Disclosures

- **Disclosures:**
  - **I have no pharma funding**
  - **When I tell a drug rep that I work on TB, s(he) politely thanks me, then leaves.**
- **Royalties:**
  - **US patent 6,291,190 B1: Molecular differences between species of the *Mycobacterium tuberculosis* complex.**
    - **Exploited in IGRA testing**
- **Generic myopia:**
  - **I generally know McGill literature better**
  - **I apologize to those working on TB in other parts of Canada whose work I have overlooked**

Outline

- Some microbiology
- Genomic epidemiology
  - Atlantic
  - Arctic
  - Pacific
- Can we use Whole Genome Sequencing in clinical TB?
  - E.g. prediction of MDR-TB?

Mycobacteria genus by multi-locus sequence analysis



Veyrier et al, BMC Evol. Biol., 2009

M. tb. complex (MTC)

M. tuberculosis

M. tb. complex (MTC)


M. tuberculosis


M. microti


Oryx bacillus


M. caprae


M. mungi

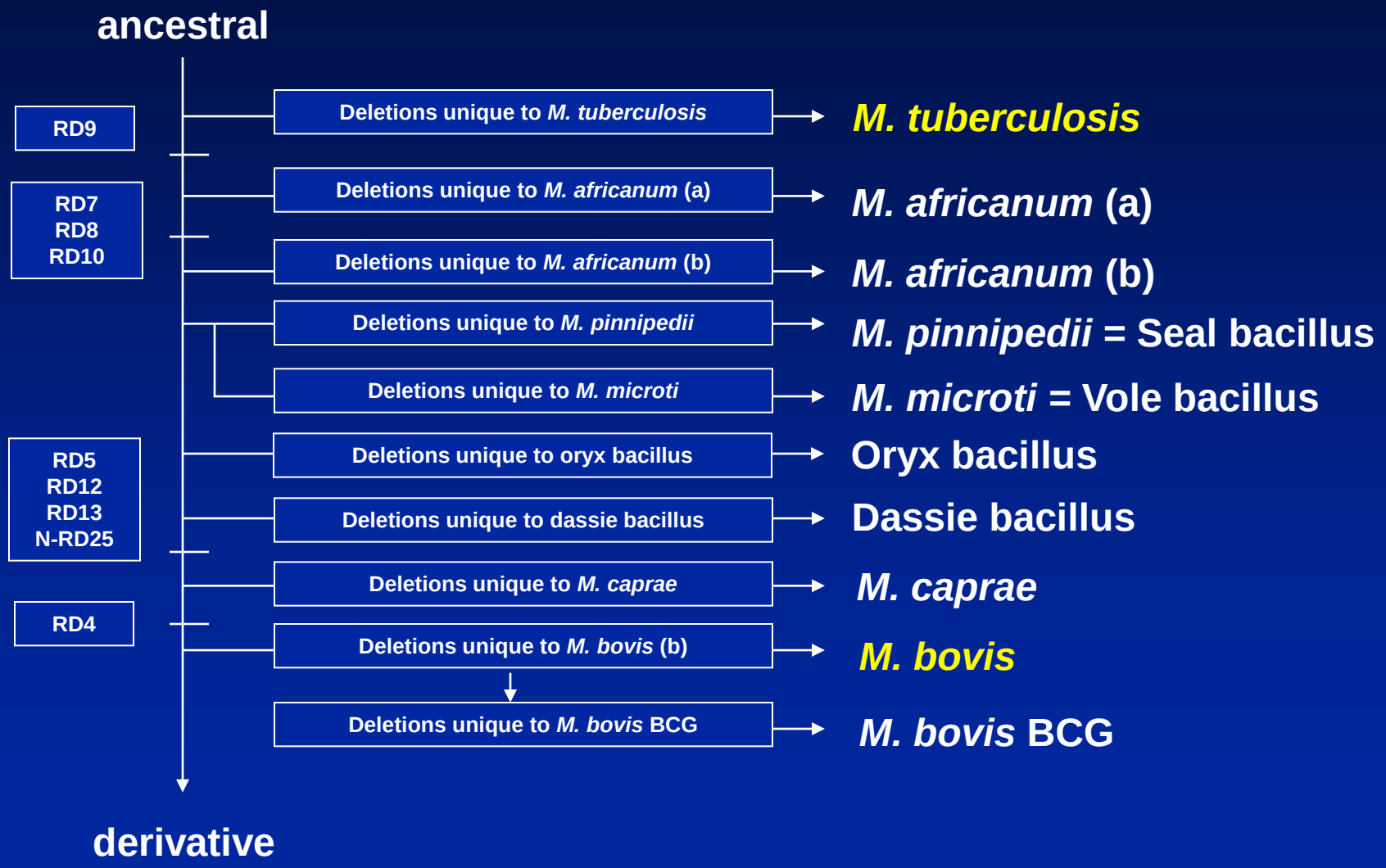

M. africanum


M. suricattae


Dassie bacillus


M. pinnipedii

Genome-based phylogeny of the MTC

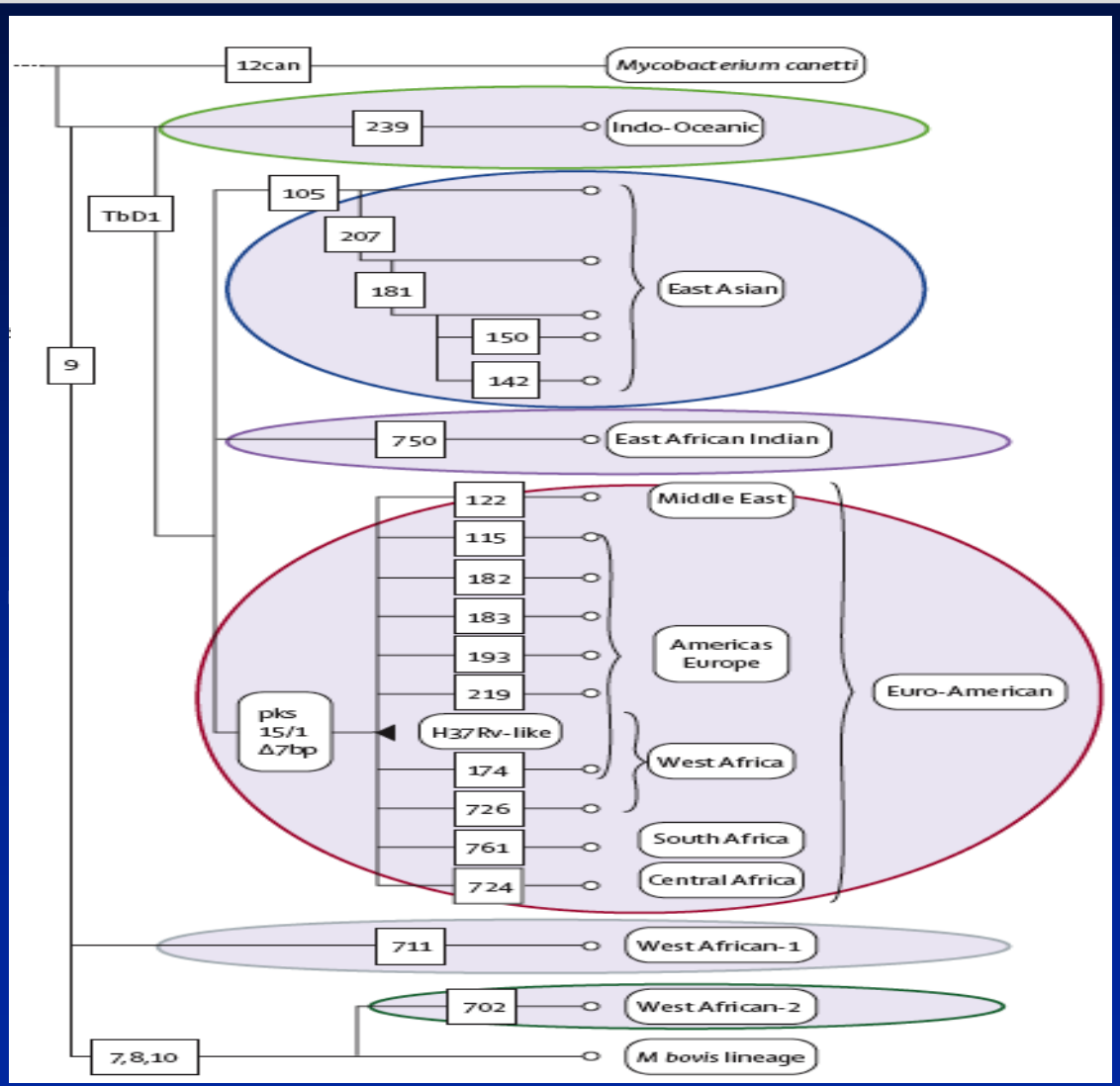


Mostowy et al., J. Bacteriology, 2005

Host-associated ecotypes of the MTC

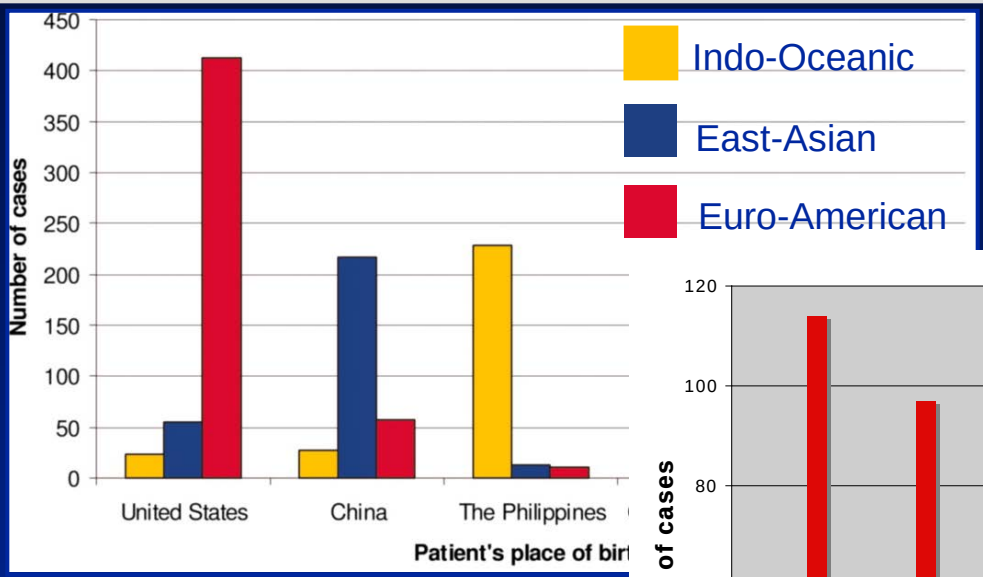
- Molecular markers for robust sub-speciation
  - All *M. pinnipedii* has same deletion pattern, regardless of provenance
- Human TB did not obviously come from cows
  - Did we give TB to goats and cows?
- Global TB control vs. One Health
- If host-pathogen association across species, what about within?

Human-associated lineages of *Mycobacterium tuberculosis*



Gagneux et al,
PNAS, 2006

Patient/strain associations

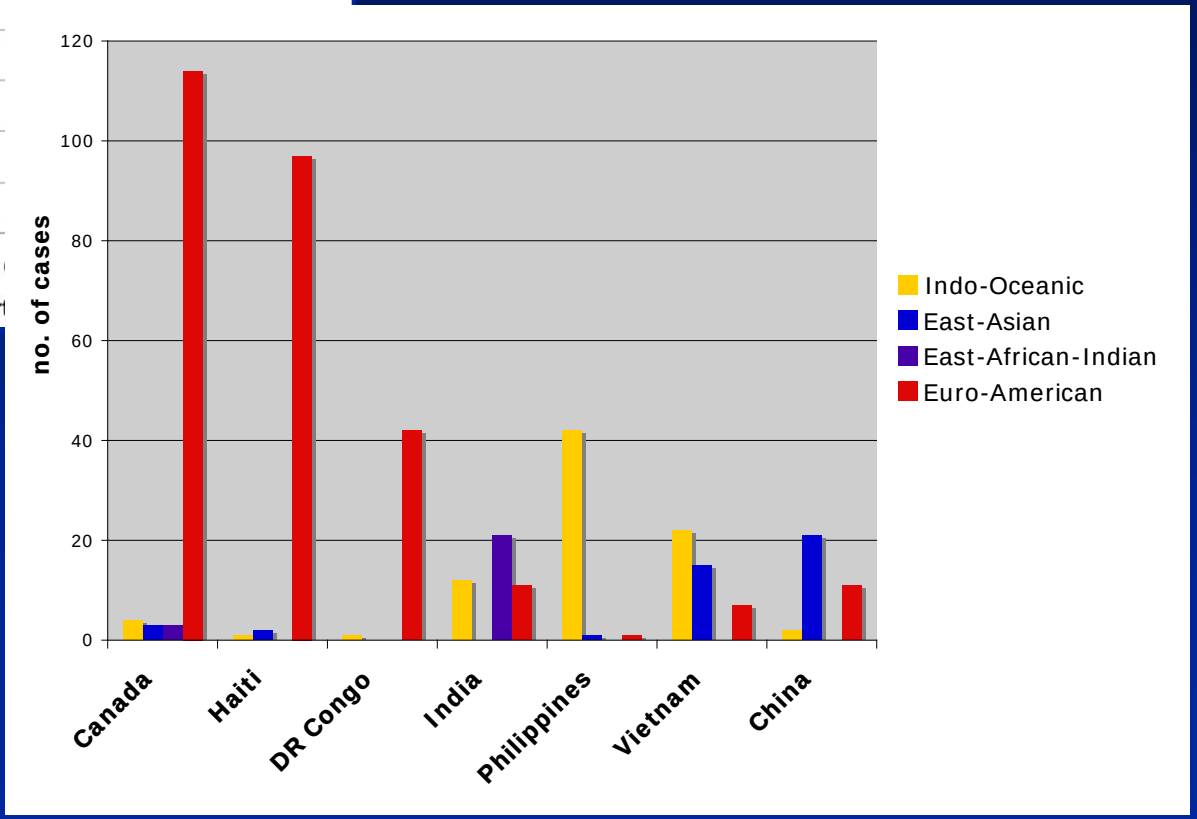


Montreal

60% of TB cases
- 7 countries

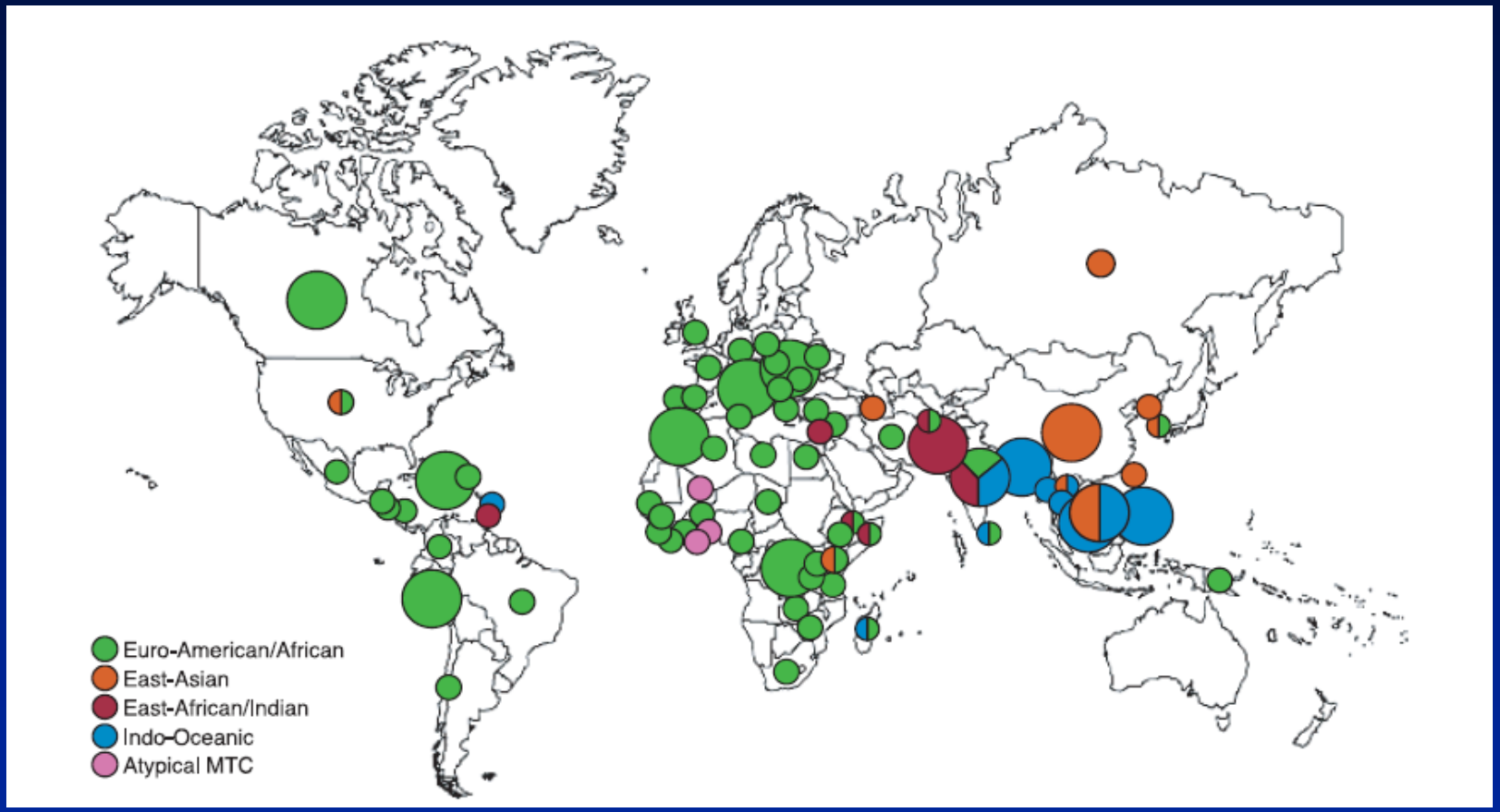
San Francisco

71% of TB cases
- 5 countries



Reed, JCM, 2009

M.tb strains & place of birth: Montreal (n = 798)



Most diversity within Africa (like humans)
Several waves of TB dispersal

Reed M et al, J. Clin Micro, 2009

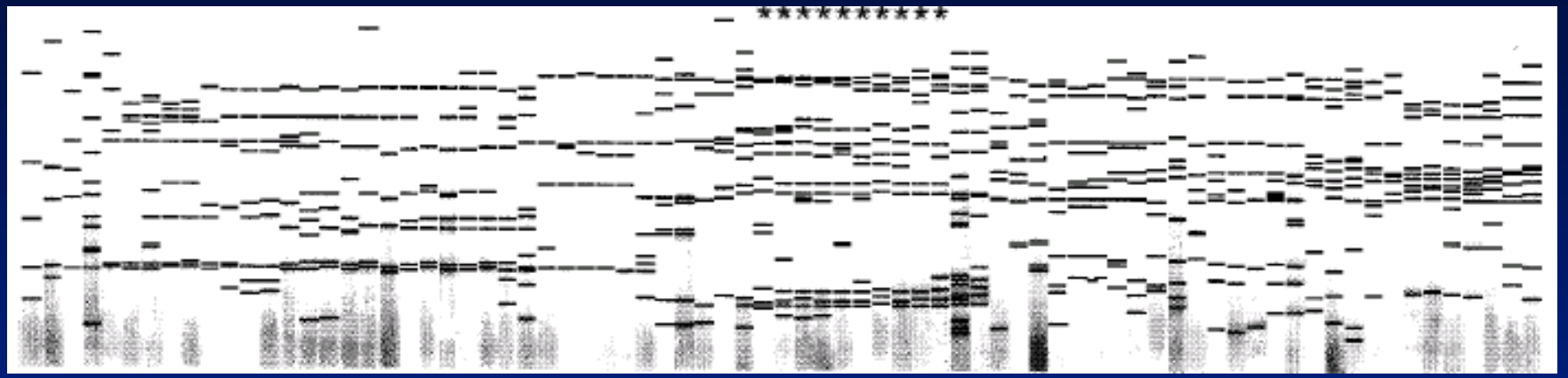
….but Canada is a big place

Reed M et al, J. Clin Micro, 2009

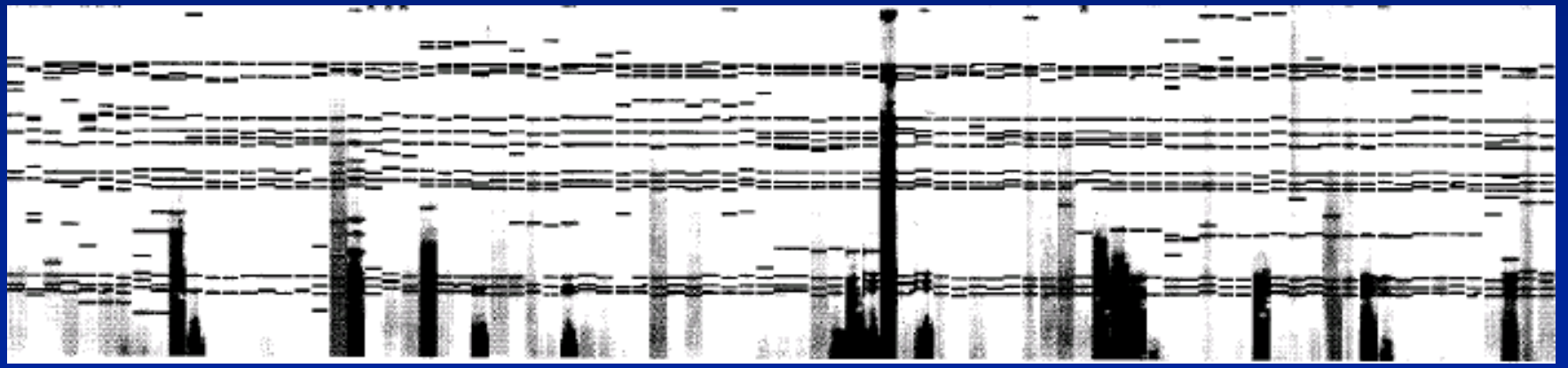
Clinical observation: Pyrazinamide mono-resistance

- Classically *M.tb* is PZA-susceptible
  - PZA-R seen with *M. bovis*
  - Due to non-synonymous SNP in *pncA*
- In Quebec, 7% of *M.tb sensu stricto* found to be PZA mono-resistant
  - A unique 8 bp deletion in *pncA* gene
- Is there a common strain?
- Any evidence of transmission?

RFLP in Quebec



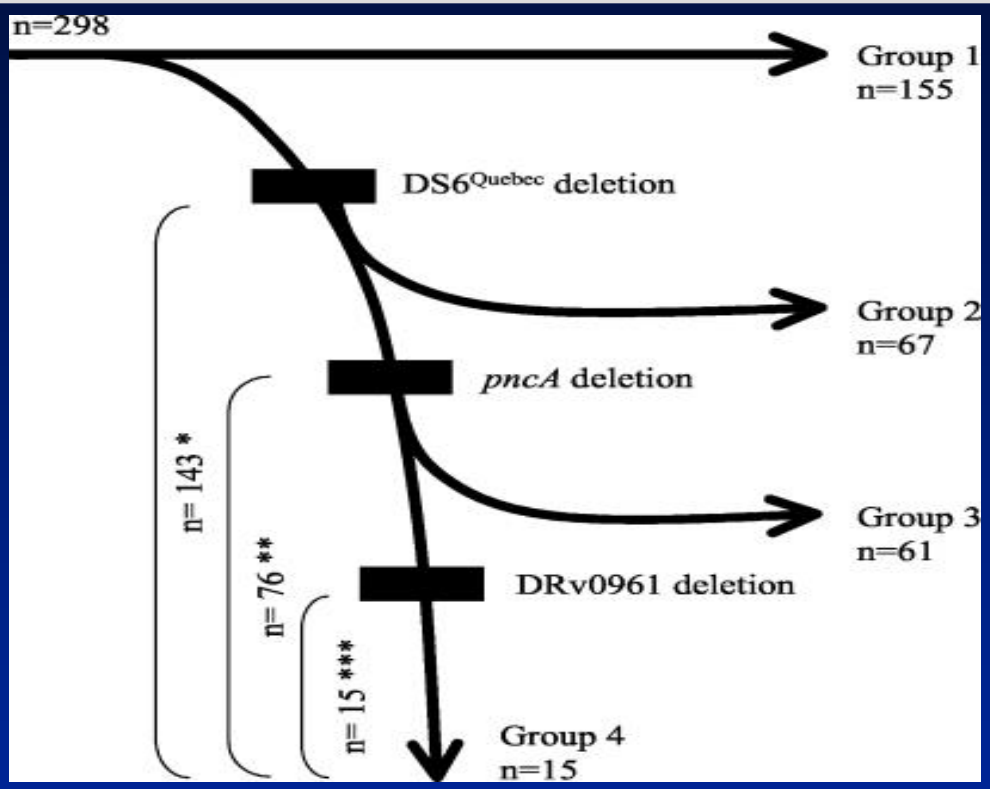
Background diversity of sensitive strains



Mono-pyrazinamide resistant strains

Nguyen et al., J. Clin Micro, 2003

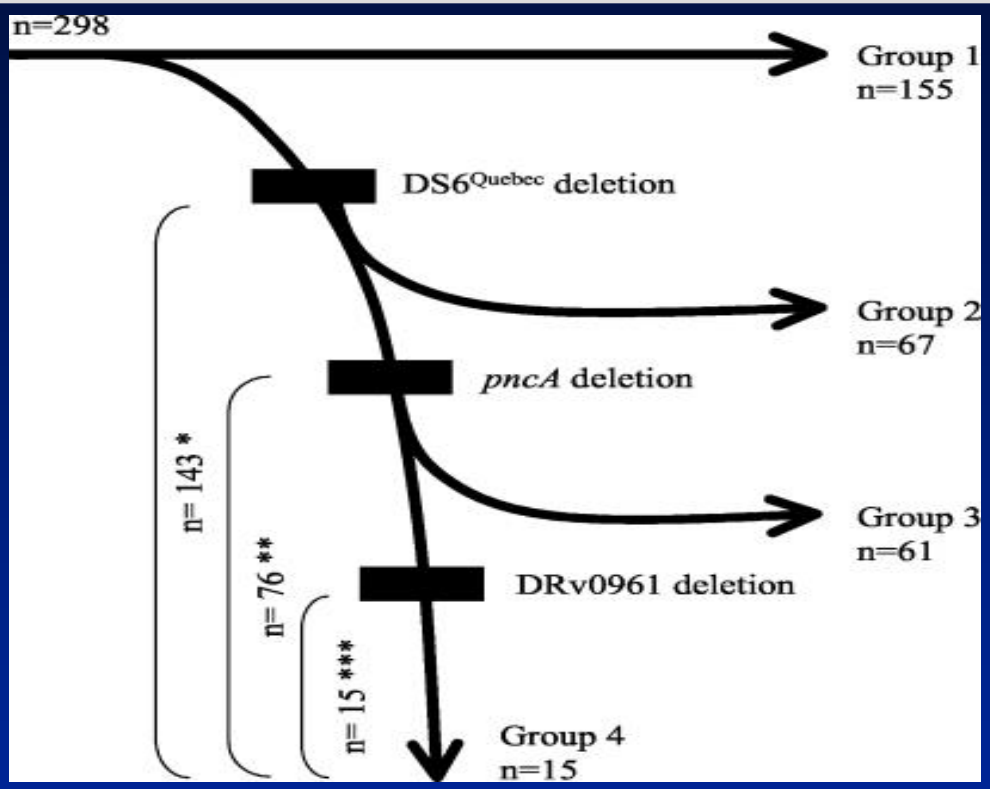
‘Quebec strain’ of *M. tuberculosis* (within Euro-American lineage)



No epidemiologic links

Nguyen D et al., J. Clin Micro, 2004

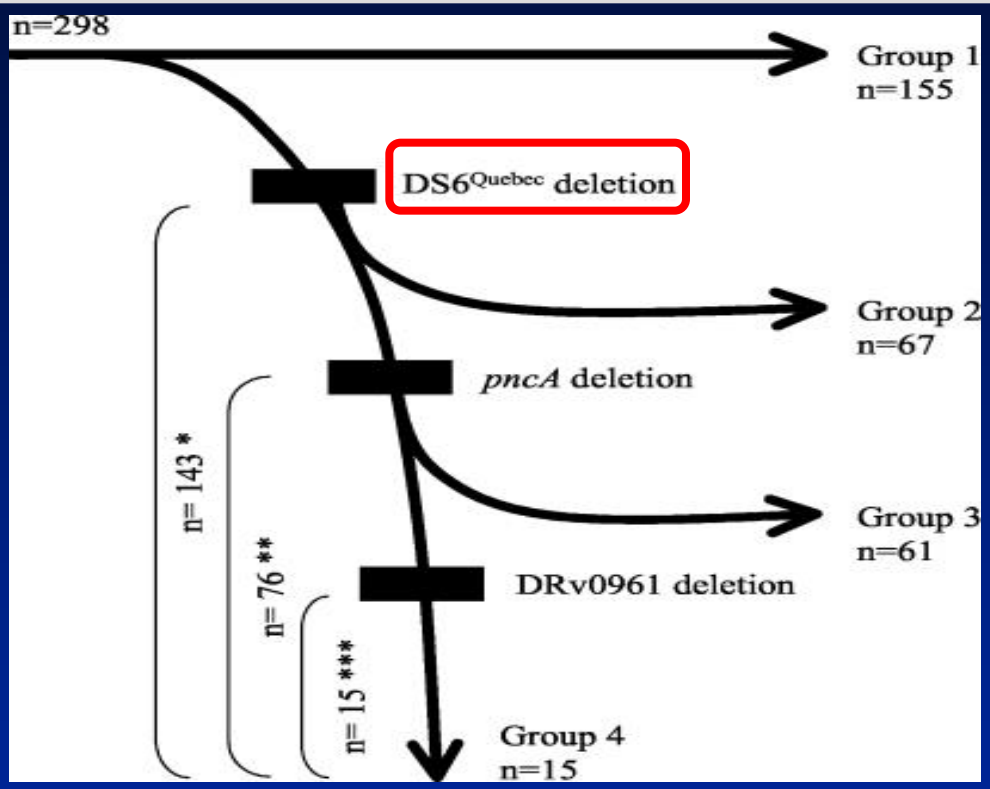
‘Quebec strain’ of *M. tuberculosis* (within Euro-American lineage)



Similar, but non-identical RFLP patterns

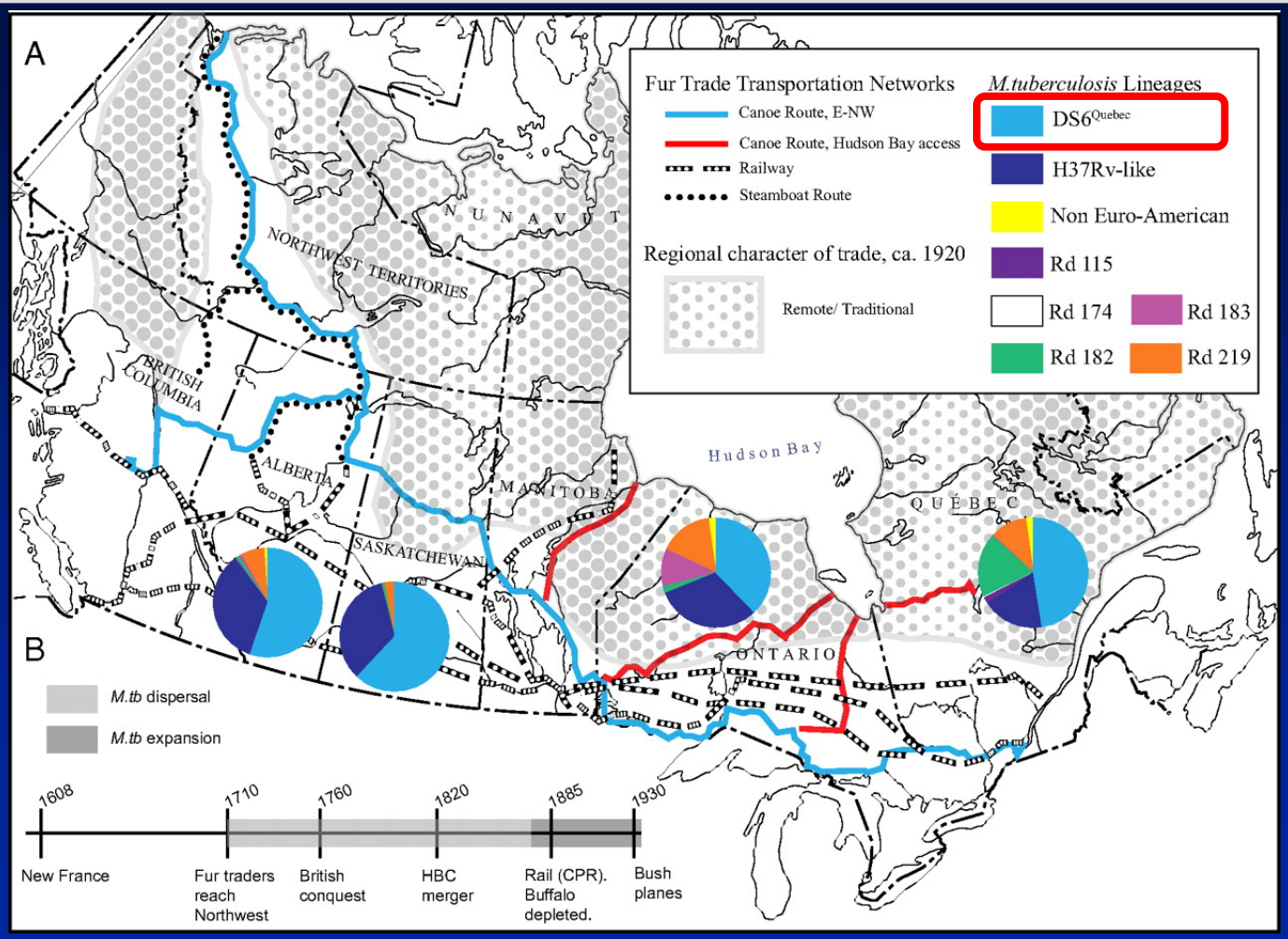
Nguyen D et al., J. Clin Micro, 2004

‘Quebec strain’ of *M. tuberculosis* (within Euro-American lineage)



Nguyen D et al., J. Clin Micro, 2004

Quebec strain of *M. tb* spread west with the Fur Trade



Pepperell C et al., PNAS, 2011

Quebec strain of *M. tb*: two phases of spread

- **Dispersal:**
  - **Quebec diversity of *M.tb* strains seen in west**
  - **Prolonged contact between traders (French) and natives (wives)**
- **Expansion:**
  - **Epidemic TB in western Canada after 1880**
  - **After Quebec-West trade mostly done**
- **The epidemic did NOT happen when the bacteria arrived; the epidemic happened when conditions changed**

Pepperell C et al., PNAS, 2011

Public Health Observation: Epidemic TB in Nunavik, 2012

Village K, Population = 933

Cases of active TB: 92 (incidence ~10%)

Culture-confirmed cases: 50 (5%)

Genotyping Kangiqsualujjuaq: MIRU

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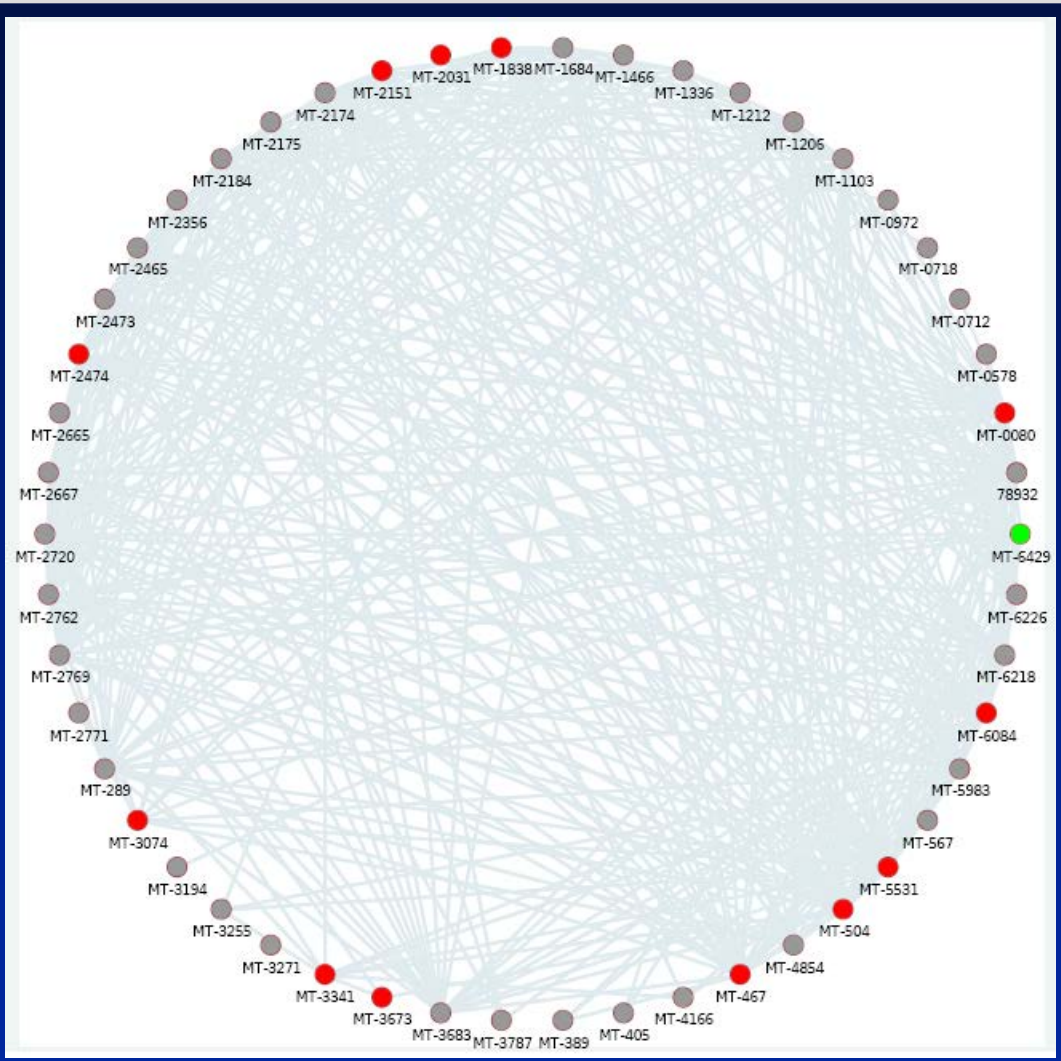
- **MIRU, 2012:**
  - **All 24 the same across 49/50 isolates (one outlier)**

Nunavik Question 1: Was it 1 outbreak?

- MIRU showed one pattern
  - Consistent with 1 outbreak
- Goal:
  - Look at public health links
  - Perform Whole Genome Sequencing (WGS) of bacteria to identify links
  - Combine the two (epi + WGS)

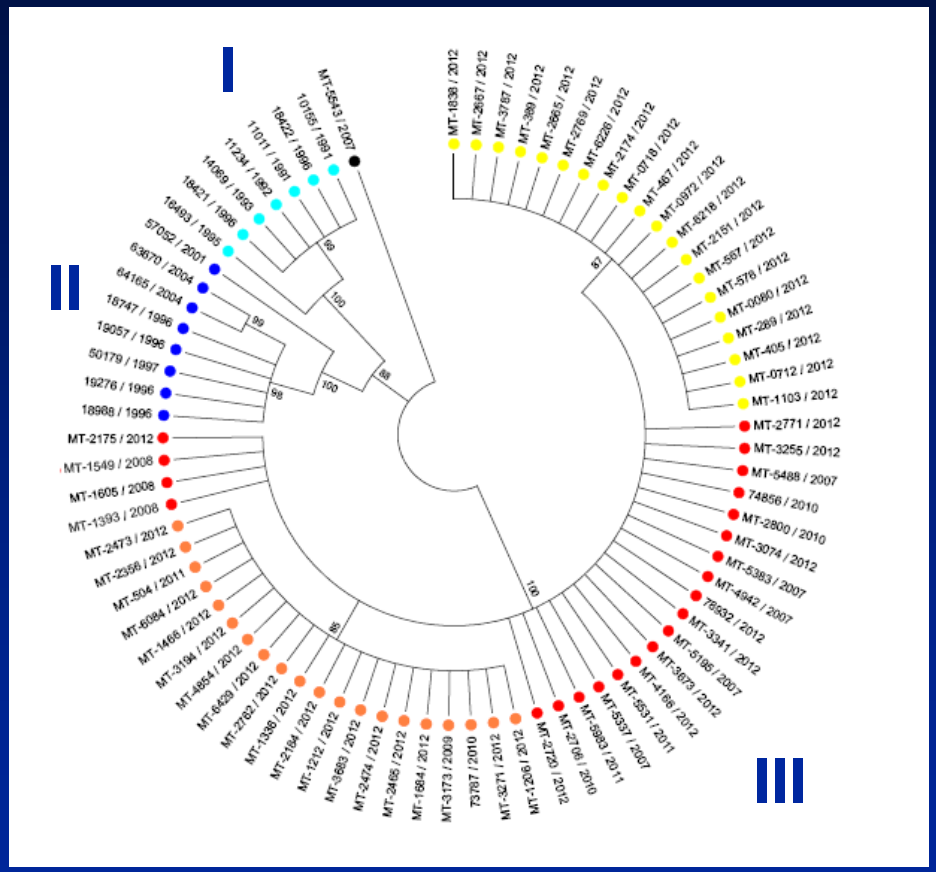
Village K: Contact data



+++ Epi links

“Everyone knows everyone”

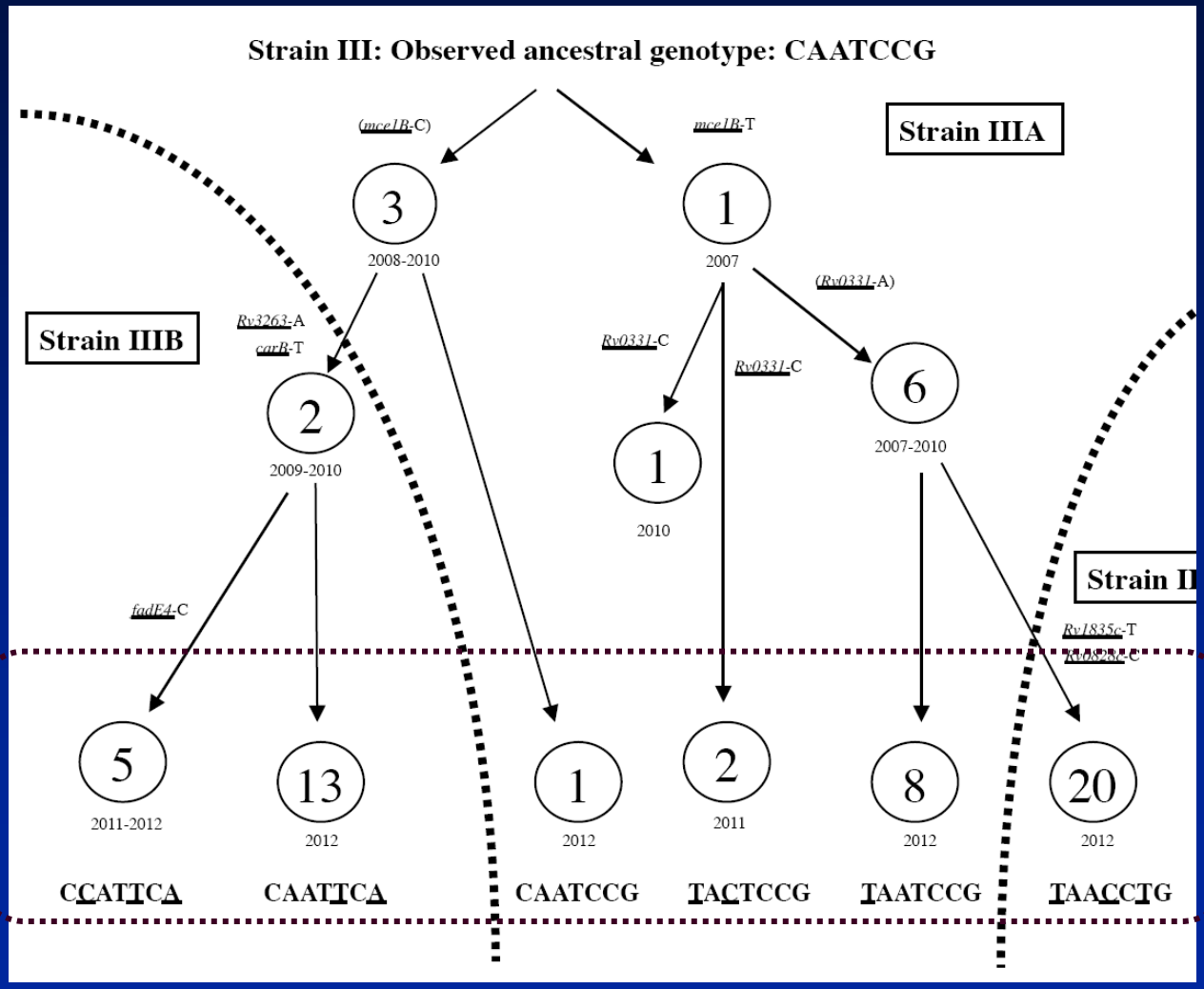
WGS of Village K 1991-2012, 78/82 sequenced



- Three strains: I, II, III (clonal replacement)
  - Strain III had 3 variants (IIIA, IIIB, IIIC)

Was it 1 event?

Combining WGS with public health files



'outbreak' was 6 (or more) events

WGS and a TB outbreak: Major findings

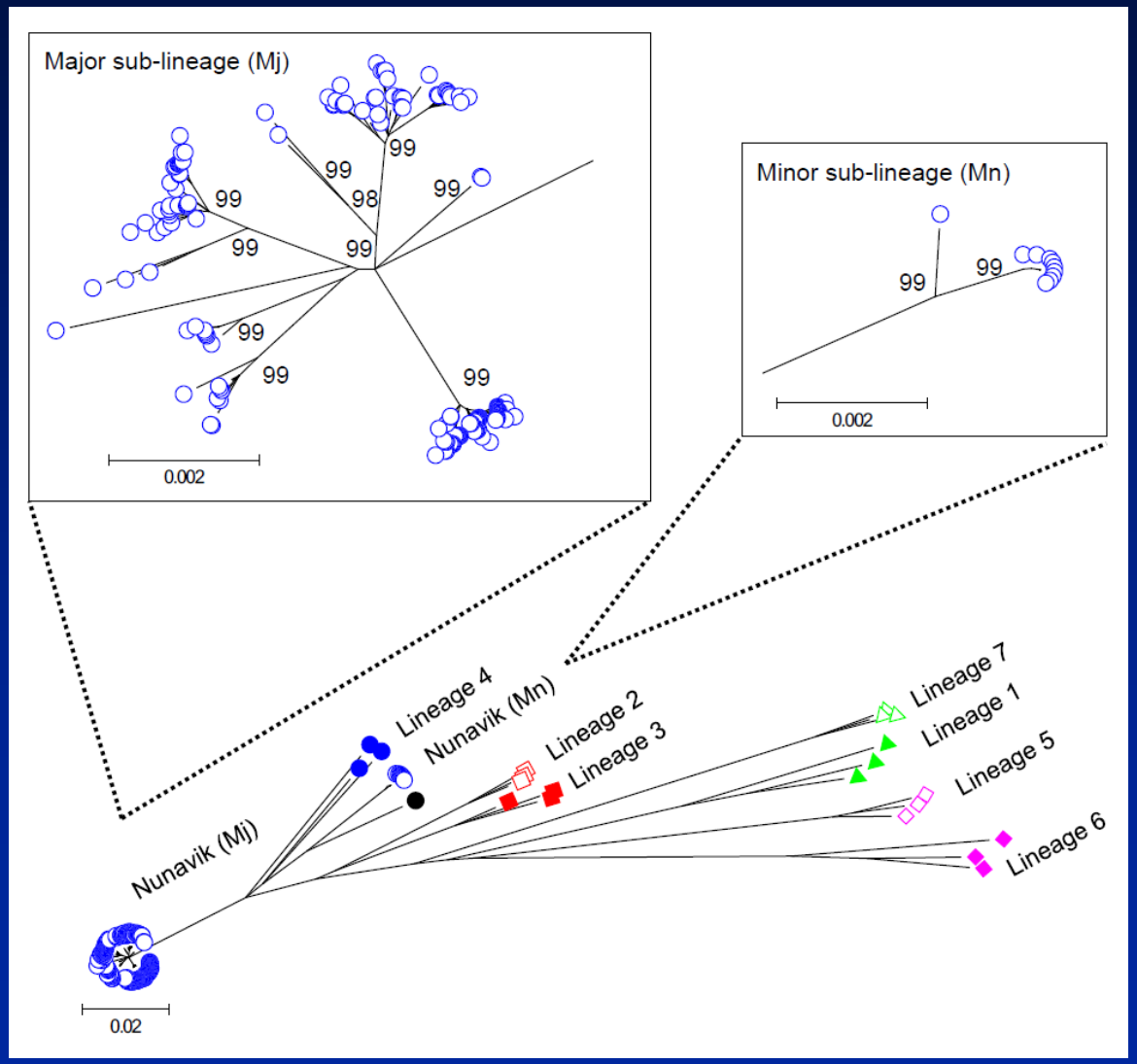
- Scale of decades:
  - Strain replacement
    - » Twice gone /Third time unlucky
- Scale of years:
  - Outbreak was multiple outbreaks
    - » Strain introduced 2007, diversified by 2012
  - No single ‘culprit’
- WGS > resolution than RFLP/MIRU
- Highest resolution: WGS + Epi

Lee et al., JID, 2015

Nunavik Question 2: Was it hypervirulent *M.tb*?

- 2012 outbreak due to a bacterium introduced in 2007
  - Where did 2007 strain come from?
- Goal:
  - Look at all villages of Nunavik
  - Perform Whole Genome Sequencing (WGS) of bacteria to infer ancestor
  - Assess bacterial evolution since its arrival in region for clues re: virulence

WGS of Nunavik 1991-2013, 163 sequences



- All Inuit isolates are Lineage 4 (Euro-American)
- NOT Quebec strain found out West
- Inuit did not bring TB from East-Asia (lineage 2)
- 153/163 descend from a single introduction

WGS of Nunavik
A century of evolution

- *M. tuberculosis* came to region circa 1919
  - From Europe or ‘European’
- Transmission primarily within villages
  - Between-village strains similar due to founder strain, not transmission
- No suggestion of hypervirulence
  - ‘lucky’ rather than ‘burly’ strain in a high-transmission environment

Lee et al, 2015, PNAS

  - Is there a transmission advantage to being a ‘wimpy’ bug?

M. tb. spread through the ages

- ***M. tuberculosis*** **came from Africa (all major lineages present)**
- ***M. tuberculosis*** **'walked' out of Africa with the paleo-migration**
- ***M. tuberculosis*** **then 'sailed' out of Europe to the Americas**
- ***M. tuberculosis*** **'canoed' and 'kayaked' across Canada**
- ***M. tuberculosis*** **has co-existed with us for a LONG time**

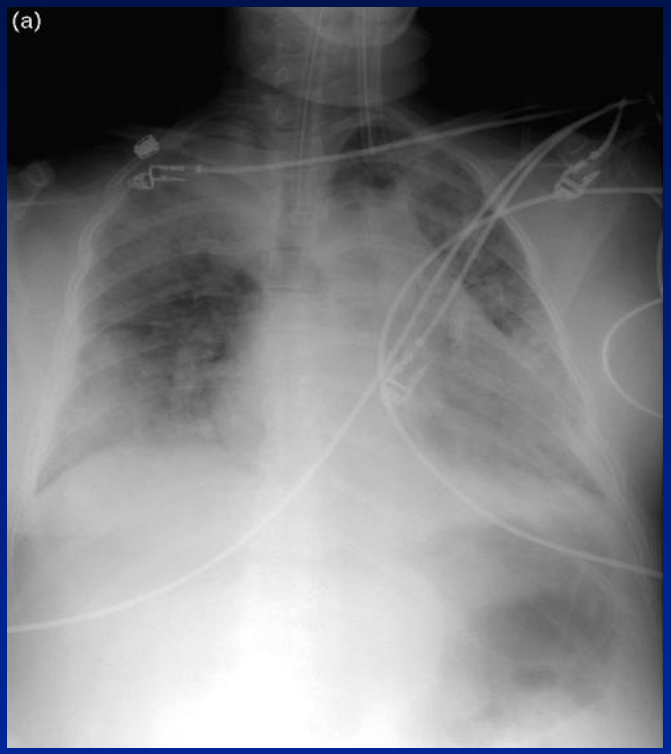
M. tb.: pathogen and symbiont

- ***M. tuberculosis*** **is a pathogen**
  - **Biomedical construct: causes disease**
  - **N.B. Disease is part of transmission cycle**
- ***M. tuberculosis*** **is a symbiont**
  - **Biological construct: symbiosis is divergent organisms that live together**

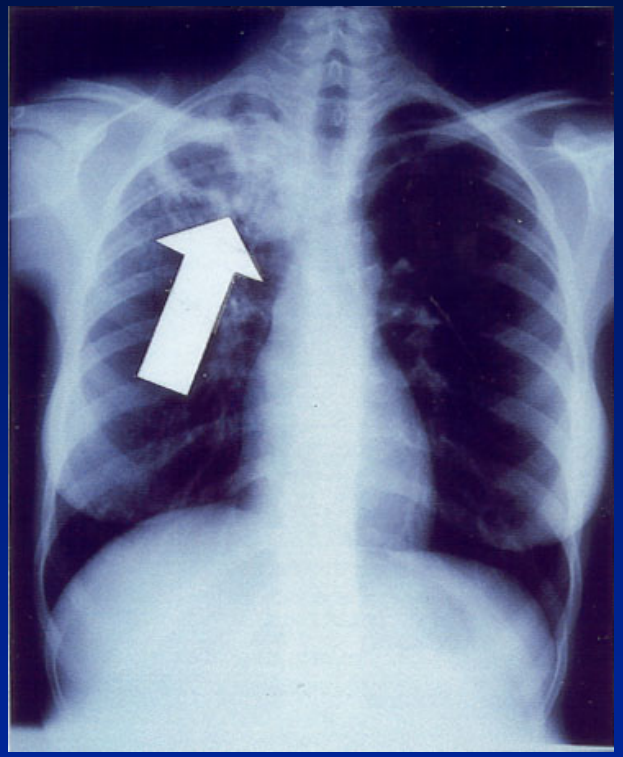
Veyrier, Dufort, Behr; Trends in Micro, 2011

- ***M. tb.*** **successfully navigates the balance between these two missions**
  - **Too virulent: kill host, ↓ transmission**
  - **Too benign: ↓ pathology, ↓ transmission**

M.tb. is an educated pathogen

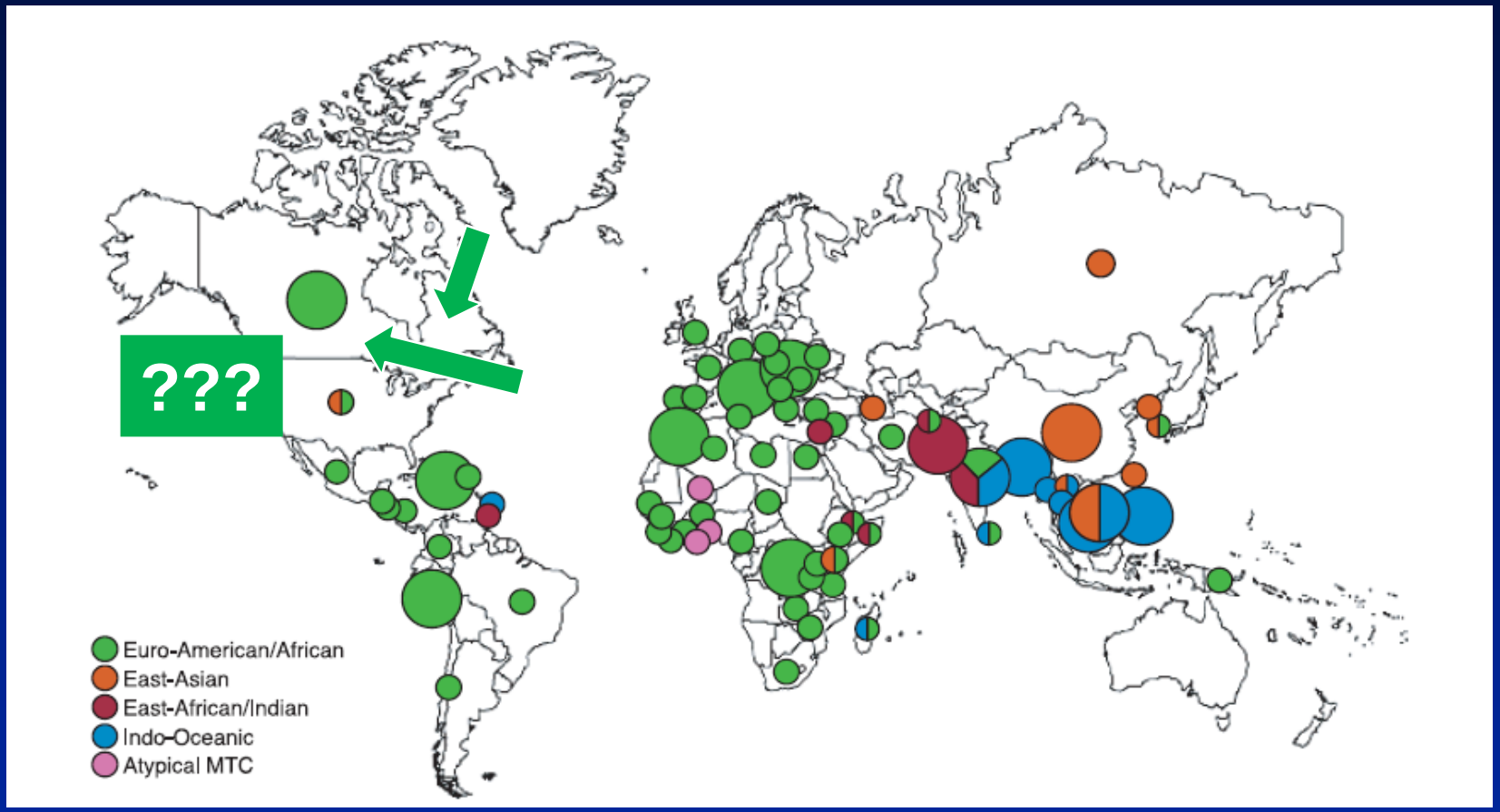


Legionella pneumophila
Diffuse, fast pathology
Uneducated Pathogen
sick host, no transmission



Mycobacterium tuberculosis
Localized, chronic pathology
Educated Pathogen
+/- sick host, transmission

M.tb strains & place of birth:



TB in the New World

- High incidence and mortality rates
  - "Virgin soil epidemic"
- Yet, fossil evidence of Pott's disease
  - E.g. pre-Colombian mummies in Peru
  - Molecular confirmation of *M.tb* DNA by PCR
- Was *M. tuberculosis* brought to Americas across Bering strait (like *Helicobacter*)?
- If not, how did people get TB 1000 years ago?

TB and the Pacific

- B.C. TB outbreak
  - Resolved by Whole Genome Sequening
    - » Gardy, NEJM, 2011
  - Isolates also belong to Euro-American lineage in secondary analysis
    - » Ford, Nature Genetics, 2013
- What about older samples?
- Paleo-DNA studies have done PCR
- Now: hybrid-capture, followed by Whole Genome Sequence (prev. used for leprosy
  - Select for MTC DNA
  - Let the sequence result tell you what you have

TB and the Pacific

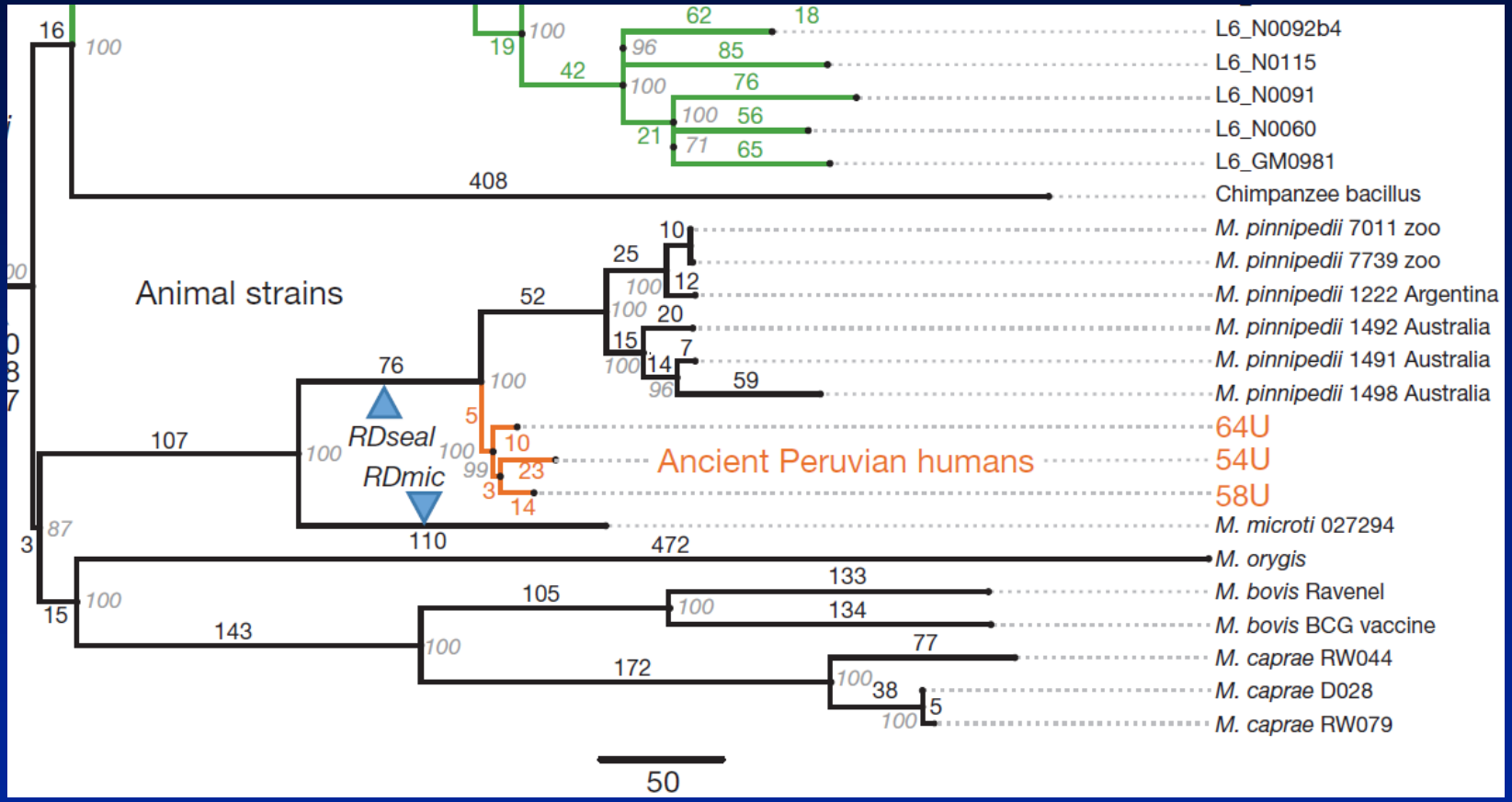
LETTER

doi:10.1038/nature13591

Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis

Kirsten I. Bos[1]*, Kelly M. Harkins[2]*, Alexander Herbig[1,3]*, Mireia Coscolla[4,5]*, Nico Weber[3], Iñaki Comas[6,7], Stephen A. Forrest[1], Josephine M. Bryant[8], Simon R. Harris[8], Verena J. Schuenemann[1], Tessa J. Campbell[9], Kerttu Majander[1], Alicia K. Wilbur[2], Ricardo A. Guichon[10], Dawnie L. Wolfe Steadman[11], Della Collins Cook[12], Stefan Niemann[13,14], Marcel A. Behr[15], Martin Zumarraga[16], Ricardo Bastida[17], Daniel Huson[3], Kay Nieselt[3], Douglas Young[18,19], Julian Parkhill[8], Jane E. Buikstra[2], Sebastien Gagneux[4,5], Anne C. Stone[2] & Johannes Krause[1,20,21]

TB and the Pacific



Bos, Nature, 2014

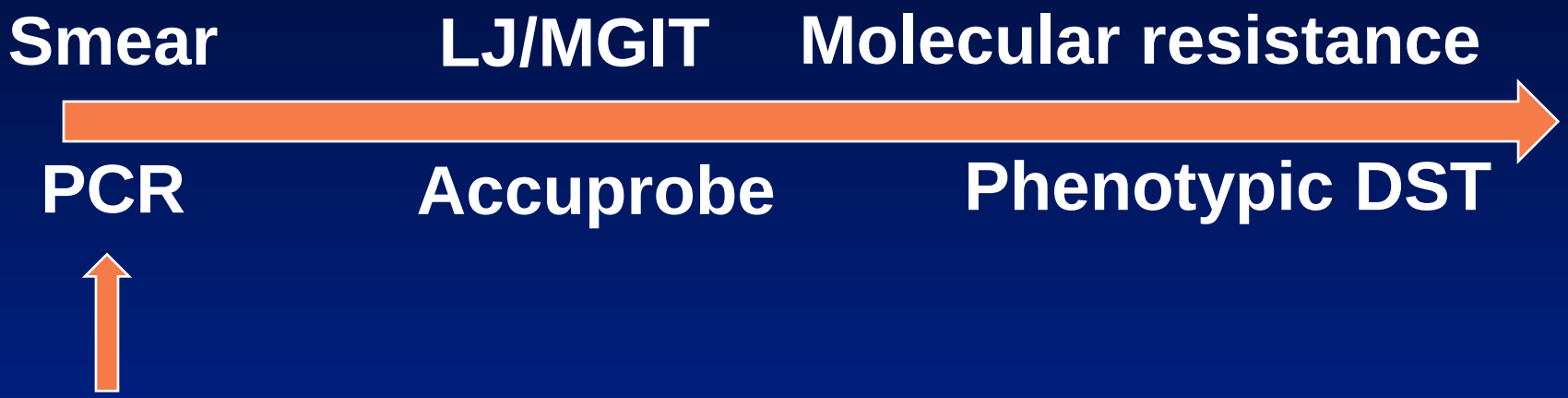
Outline - revisited

- Epidemiology of TB in Canada
  - TB previously from Europe
    - » Dissemination, then expansion
    - » TB exploits social opportunities
  - TB presently from sites of immigration
- Methods
  - Whole Genome Sequencing is clearly best for epidemiology
  - WGS can be used to diagnose cause of TB in pre-Colombian mummies
  - But can it be clinically useful?

WGS visits the clin TB lab

Where could WGS be useful?

WGS visits the clin TB lab



WGS on sample

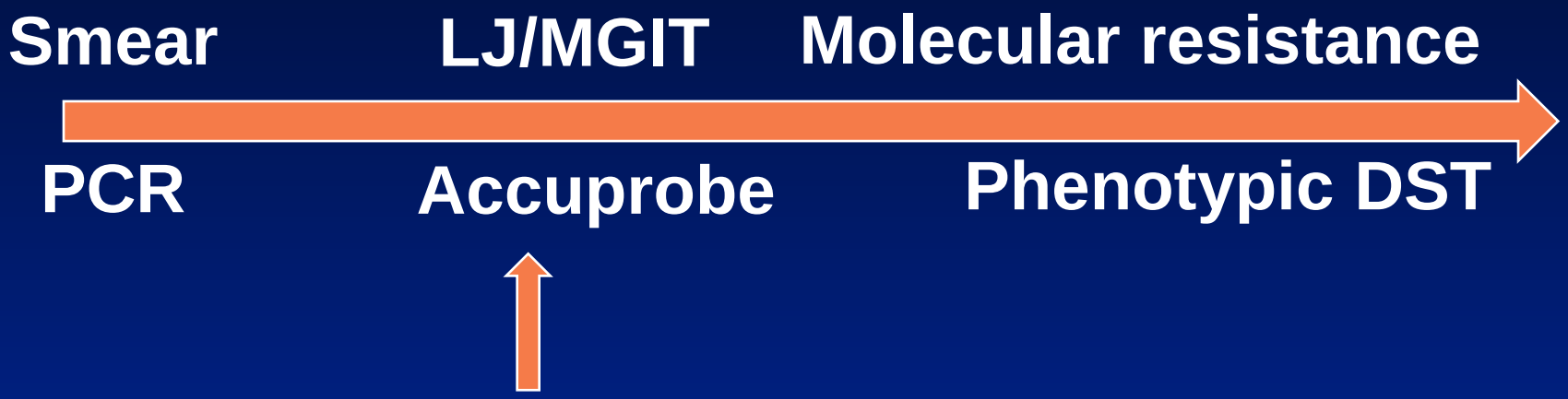
Lots of negatives

Lots of other DNA

Need $$$ enrichment

Reviewed in Lee & Behr, Therapeutic Advances in ID, 2016

WGS visits the clin TB lab



WGS on + culture

Speciation – nice

More rapid DST?

Reviewed in Lee & Behr, Therapeutic Advances in ID, 2016

DST prediction by WGS

- Operating parameters
  - Sensitivity/Specificity:
    - » 83% / 98.5% - Bradley, Nature Comm, 2015
  - Overall accuracy:
    - » 93% - Pankhurst, Lancet Resp Med, 2016

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REVIEW ARTICLE

Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement

J. Domínguez,* E. C. Boettger,† D. Cirillo,‡ F. Cobelens,§ K. D. Eisenach,¶ S. Gagneux,#
D. Hillemann,** R. Horsburgh,†† B. Molina-Moya,* S. Niemann,‡‡ E. Tortoli,§§ A. Whitelaw,¶¶
C. Lange;##***††† for the TBNET and RESIST-TB networks

DST prediction by WGS

The emergence of drug-resistant strains of *Mycobacterium tuberculosis* is a challenge to global tuberculosis (TB) control. Although culture-based methods have been regarded as the gold standard for drug susceptibility testing (DST), molecular methods provide rapid information on mutations in the *M. tuberculosis* genome associated with resistance to anti-tuberculosis drugs. We ascertained consensus on the use of the results of molecular DST for clinical treatment decisions in TB patients. This document has been developed by TBNET and RESIST-TB groups to reach a consensus about reporting standards in the clinical use of molecular DST results. Review of the available literature and the search for evidence included hand-searching journals and searching electronic databases. The panel identified single nucleotide mutations in genomic regions of *M. tuberculosis* coding for *kat*G, *inh*A, *rpo*B, *emb*B, *rrs*, *rps*L and *gyr*A that are likely related to drug resistance in vivo. Identification of any of these mutations in clinical isolates of *M. tuberculosis* has implications for the management of TB patients, pending the results of in vitro DST. However, false-positive and false-negative results in detecting resistance-associated mutations in drugs for which there is poor or unproven correlation between phenotypic and clinical drug resistance complicate the interpretation. Reports of molecular DST results should therefore include specific information on the mutations identified and provide guidance for clinicians on interpretation and on the choice of the appropriate initial drug regimen.

KEY WORDS: clinician guidance; interpretation; molecular methods

Mycobacterium tuberculosis: a TBNET/RESIST-TB consensus statement

J. Domínguez,* E. C. Boettger,† D. Cirillo,‡ F. Cobelens,§ K. D. Eisenach,¶ S. Gagneux,#
D. Hillemann,** R. Horsburgh,†† B. Molina-Moya,* S. Niemann,‡‡ E. Tortoli,§§ A. Whitelaw,¶¶
C. Lange;##***††† for the TBNET and RESIST-TB networks

So is WGS faster than DST?

"Unadjusted median time from MGIT positivity to DST reporting was 25 days (IQR 14–32)…similarly, full WGS based reports were available in 31 days (IQR 21-60)"

"WGS processing delays were driven by sample batching for sequencing and delays in sharing seque ncing data….The time delay would be minimised in high-throughput labs" Potentially 8 days

If MGIT takes 10 days to flag:

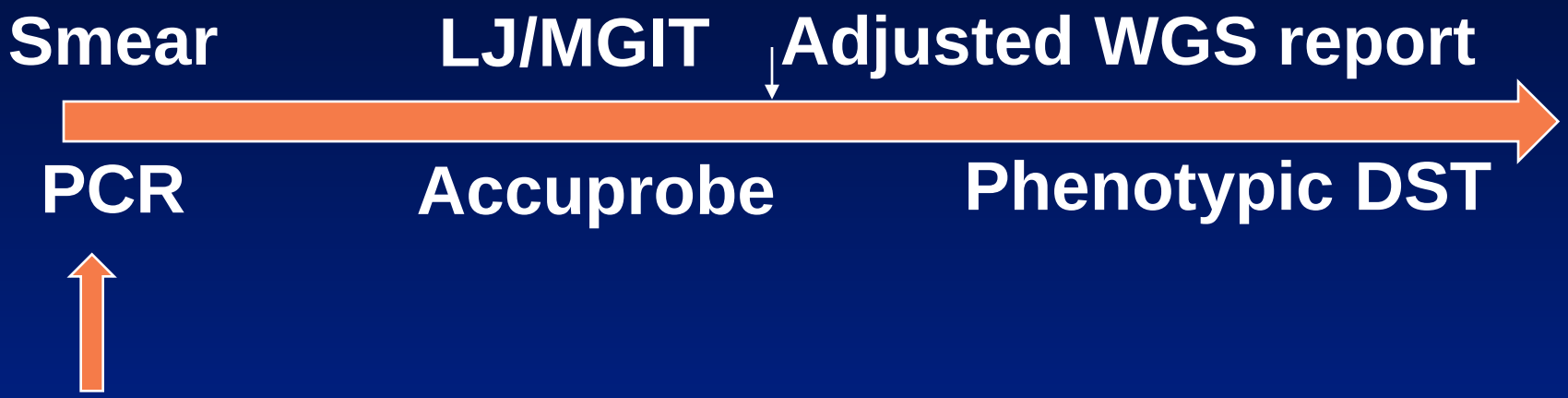
Full DST is on day 35

WGS-based prediction feasible by day 18

Pankhurst, Lancet Resp Med, 2016

WGS visits the clin TB lab

WGS visits the clin TB lab



What does the MD prescribe here?

Can the MD wait 18 days to start TB Rx?

WGS and global MDR-TB

Canada: 19 cases in 1376, 2014

WHO: 480,000 MDR-TB cases (210,000 deaths)
DST: 12% new cases, 58% prev. treated

WGS for clinical TB

- Proof-of-concept here
- Reasonably good for calling resistance
  - Not reliable enough yet for calling susceptible
- If sensitivity does not reach 100%, we will still need phenotypic DST
  - WGS as added, not replacement test
- Canada might be a hard place to prove relevance

Concluding thoughts

- MTC: Mammalian mycobacteria
  - New names good for challenging fellows on Royal College exams
- Molecular epidemiology
  - TB in Canada mostly reactivation
  - Pockets of ongoing spread due to bacteria here for 1-3 centuries
- WGS in clinical lab
  - Technology almost ready
  - Operating parameters? Need?

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www.mcgill.ca/molepi

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