Genomic epidemiology:
what is it? and how will it inform public health policy?

Nigel French

Infectious Disease Research Centre, Massey University
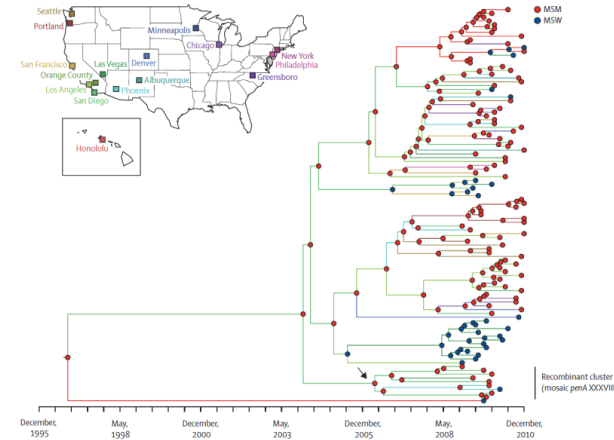
Infectious Disease mini-symposium
September 2014

Collaborating Centre

Genomic epidemiology

- Epidemiology informed by whole genome sequencing
  - NGS technology
  - Host and/or pathogen
- Used for communicable and non-communicable diseases
  - GWAS
- Number of studies increased in last 5-6 years
- Lot of work in progress

Recent articles

Research

EMERGING INFECTIOUS DISEASES®

Genomic Epidemiology of *Salmonella enterica* Serotype Enteritidis based on Population Structure of Prevalent Lineages

Xiangyu Deng✉ , Prerak T. Desai, Henk C. den Bakker, Matthew Mikoleit, Beth Tolar, Eija Trees, Rene S. Hendriksen, Jonathan G. Frye, Steffen Porwollik, Bart C. Weimer, Martin Wiedmann, George M. Weinstock, Patricia I. Fields[1], and Michael McClelland[1]

OPEN ACCESS Freely available online

PLOS | PATHOGENS

Whole Genome Sequencing Reveals Local Transmission Patterns of *Mycobacterium bovis* in Sympatric Cattle and Badger Populations

Roman Biek[1,9], Anthony O'Hare[1,9], David Wright[2,3], Tom Mallon[2], Carl McCormick[2], Richard J. Orton[1], Stanley McDowell[2], Hannah Trewby[1], Robin A. Skuce[2], Rowland R. Kao[1]*

Population genomics of post-vaccine changes in pneumococcal epidemiology

Nicholas J Croucher[1,2], Jonathan A Finkelstein[3,4], Stephen I Pelton[5], Patrick K Mitchell[1], Grace M Lee[3,6,7], Julian Parkhill[2], Stephen D Bentley[2,8,10], William P Hanage[1,10] & Marc Lipsitch[1,9,10]

Recent articles

Genomic epidemiology of the *Escherichia coli* O104:H4 outbreaks in Europe, 2011

www.pnas.org/cgi/doi/10.1073/pnas.1121491109

Yonatan H. Grad[a,b], Marc Lipsitch[b,c], Michael Feldgarden[d], Harindra M. Arachchi[d], Gustavo C. Cerqueira[d], Michael FitzGerald[d], Paul Godfrey[d], Brian J. Haas[d], Cheryl I. Murphy[d], Carsten Russ[d], Sean Sykes[d], Bruce J. Walker[d], Jennifer R. Wortman[d], Sarah Young[d], Qiandong Zeng[d], Amr Abouelleil[d], James Bochicchio[d], Sara Chauvin[d], Timothy DeSmet[d], Sharvari Gujja[d], Caryn McCowan[d], Anna Montmayeur[d], Scott Steelman[d], Jakob Frimodt-Møller[e,f], Andreas M. Petersen[f,g], Carsten Struve[f], Karen A. Krogfelt[f], Edouard Bingen[h,i], François-Xavier Weill[j], Eric S. Lander[d,k,l,1], Chad Nusbaum[d], Bruce W. Birren[d], Deborah T. Hung[a,d,m,n,2], and William P. Hanage[b,1,2]

Genomic epidemiology of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime in the USA: a retrospective observational study

Lancet Infect Dis 2014; 14: 220–26

Yonatan H Grad, Robert D Kirkcaldy, David Trees, Janina Dordel, Simon R Harris, Edward Goldstein, Hillard Weinstock, Julian Parkhill, William P Hanage, Stephen Bentley, Marc Lipsitch

Distinguishable Epidemics of Multidrug-Resistant *Salmonella* Typhimurium DT104 in Different Hosts

27 SEPTEMBER 2013   VOL 341   **SCIENCE**

A. E. Mather,[1] S. W. J. Reid,[2]‡ D. J. Maskell,[3] J. Parkhill,[1] M. C. Fookes,[1] S. R. Harris,[1] D. J. Brown,[4] J. E. Coia,[4] M. R. Mulvey,[5] M. W. Gilmour,[5]* L. Petrovska,[6] E. de Pinna,[7] M. Kuroda,[8] M. Akiba,[9] H. Izumiya,[10] T. R. Connor,[1]† M. A. Suchard,[11] P. Lemey,[12] D. J. Mellor,[13] D. T. Haydon,[13] N. R. Thomson[1]‡

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Recent articles: review and commentary

Evolutionary epidemiology: preparing for an age of genomic plenty

O. G. Pybus[1], C. Fraser[2] and A. Rambaut[3]

PHILOSOPHICAL TRANSACTIONS —OF— THE ROYAL SOCIETY B

Bacterial genomes in epidemiology— present and future

Nicholas J. Croucher[1], Simon R. Harris[2], Yonatan H. Grad[1,3] and William P. Hanage[1]

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Applications

- Transmission
  - Estimating Ro
  - Transmission in social networks
  - Anthroponoses, zoonoses (incl reverse zoonoses)

- Host switching
  - Incl. Zoonoses

- Phenotype:genotype relationships
  - Broad definition of phenotype (host association, seasonality, carbon source utilisation)

- Geographical movements and incursion
  - Number and origin of introductions

- Evolution
  - Impact of interventions (vaccination)
  - Changes in virulence
  - Antimicrobial resistance (Debbie Williamson)

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Whole-Genome Sequencing and Social-Network Analysis of a Tuberculosis Outbreak

Gardy et al, 2011 New Engl J Med, 364, 730-739

- 3 year outbreak of TB in British Colombia
  - In community struggling with alcoholism, drug abuse and transient housing status
- Typed isolates using mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR)
  - Evidence that outbreak was due to a single clone
- Traditional contact tracing could not identify a source

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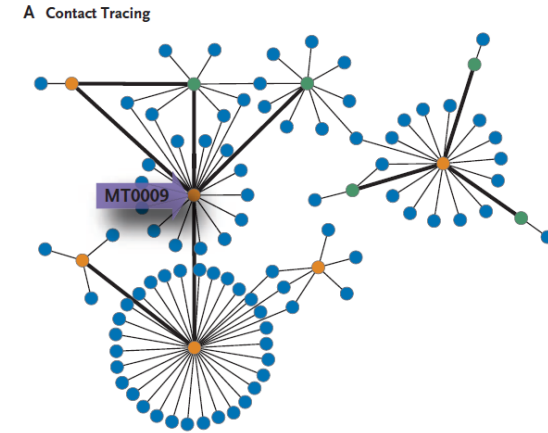
- Whole genome sequences for 36 isolates
- Found two distinct lineages
- Identical MIRU-VNTR types
- Suggested two concomitant outbreaks
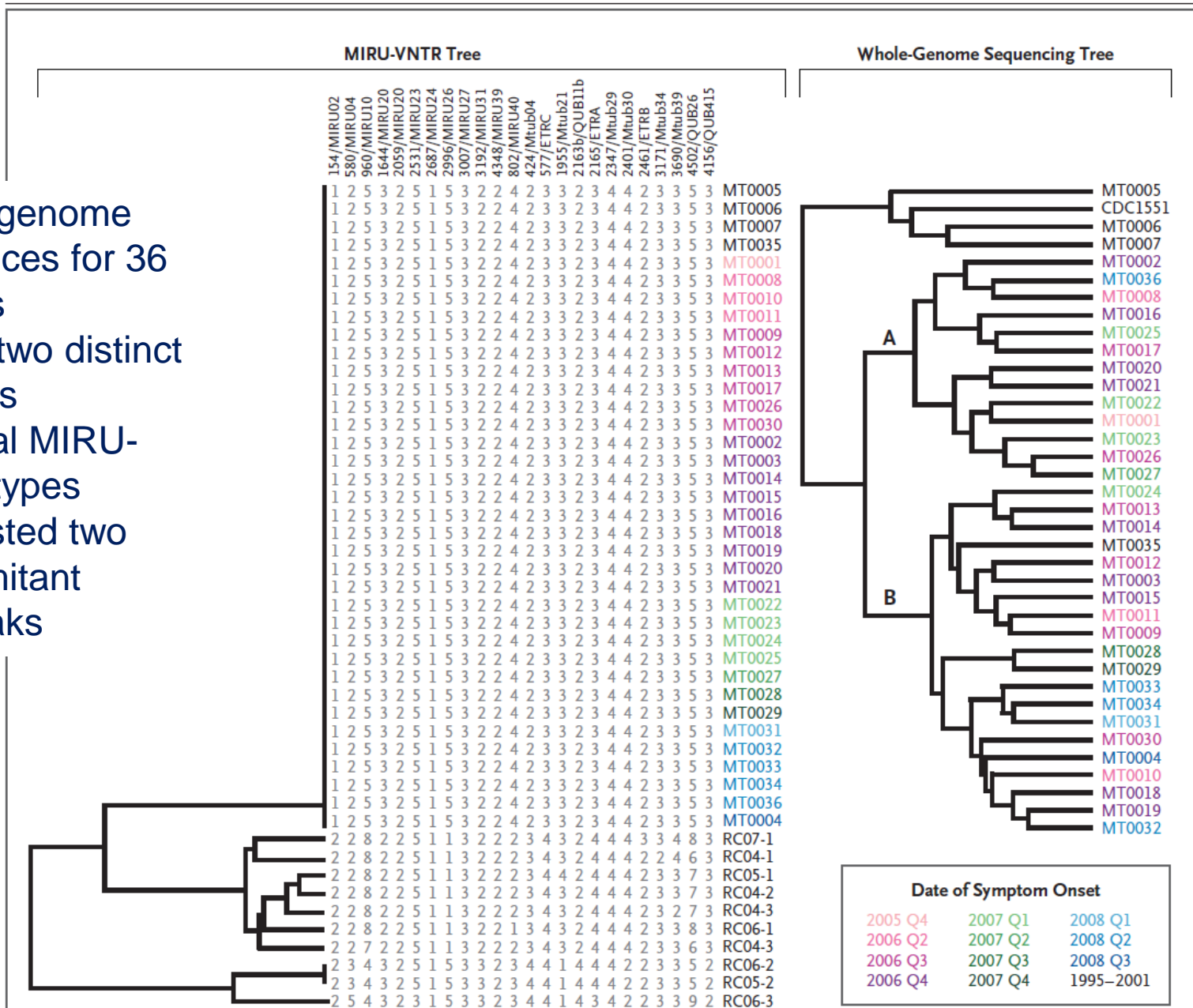
Figure 3. Hierarchical Clustering of Patterns of Mycobacterial Interspersed Repetitive Unit–Variable Number Tandem Repeats (MIRU-VNTRs) and Whole-Genome–Derived Genetic Distances of 36 *Mycobacterium tuberculosis* Isolates.
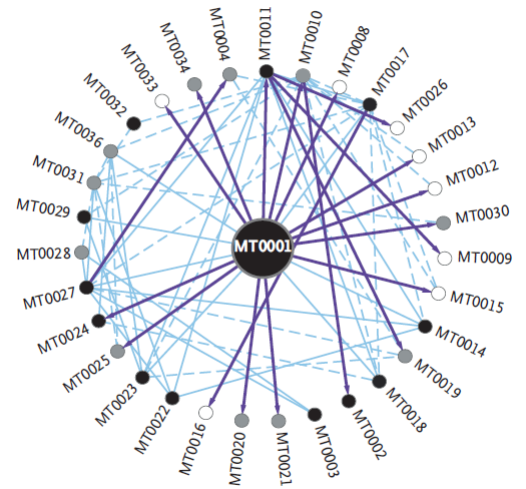
Social network analysis

- Interviewed cases and constructed a social network to look for key:
  - Persons
  - Places
  - Behaviours
- Highlighted the most probable source case

Combining phylogeny and SNA

- Brought phylogeny and social network together
- MT001 = most probable source for first outbreak
  - "superspreader"
  - Most cases unconnected with each other, but had contact with MT001

A MIRU-VNTR and Social-Network Analysis

B Whole-Genome Sequencing and Social-Network Analysis
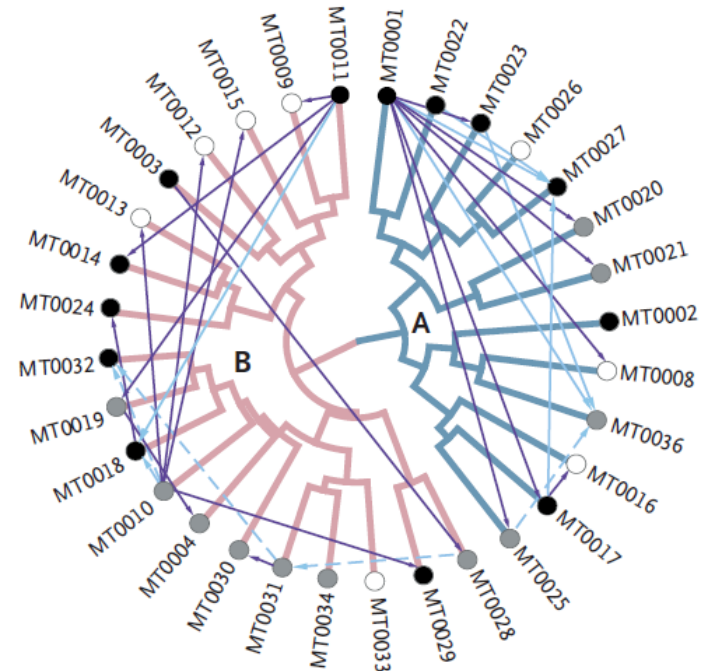
Truth and error (Croucher et al)

Table 1. Summary of the processes that generate variation in genomic alignments in epidemiology. It should be noted that of these, point mutations inherited clonally should form the basis of any phylogenetic analysis. ← See Tim Vaughan's talk

	processes
true variation	point mutation
	insertions and deletions (tandem repeats/homopolymers, etc)
	homologous recombination
	mobile genetic elements
false-positives	SNPs generated *in vitro*
	mapping errors (frequently associated with repeat sequences)
	systematic sequencing errors
	'ectopic' mapping—reads mapped to paralogous rather than homologous loci in reference.
	poor filters
false-negatives	failure to assemble repetitive regions
	systematic sequencing errors
	poor filters
	distantly related reference sequence, such that variants cannot be called throughout the genome

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Genomic epidemiology of *Campylobacter* spp. in New Zealand
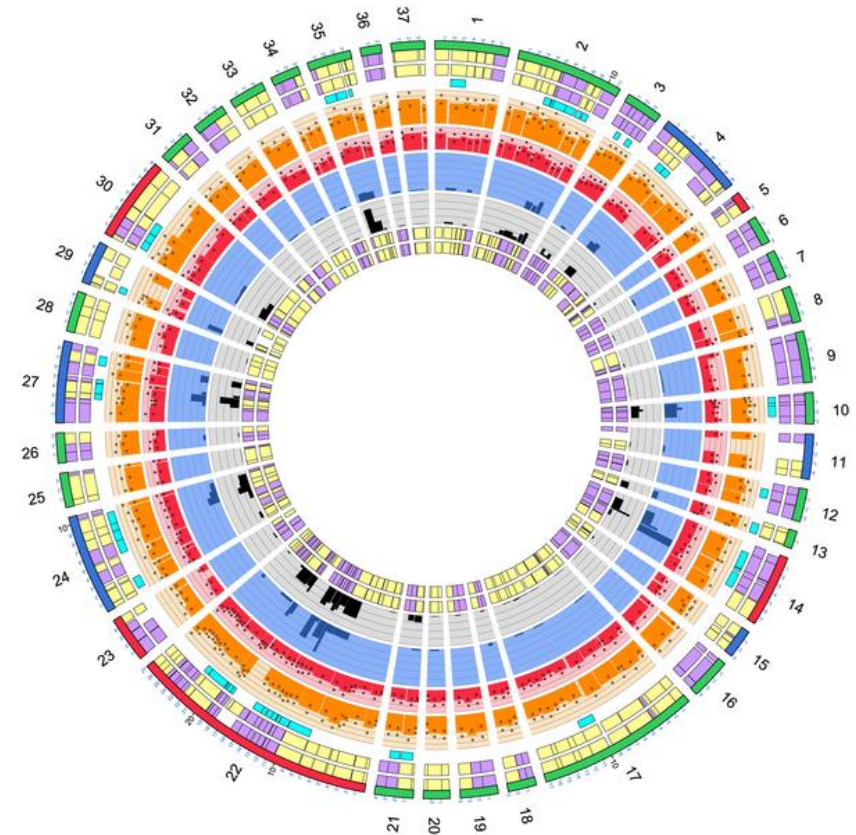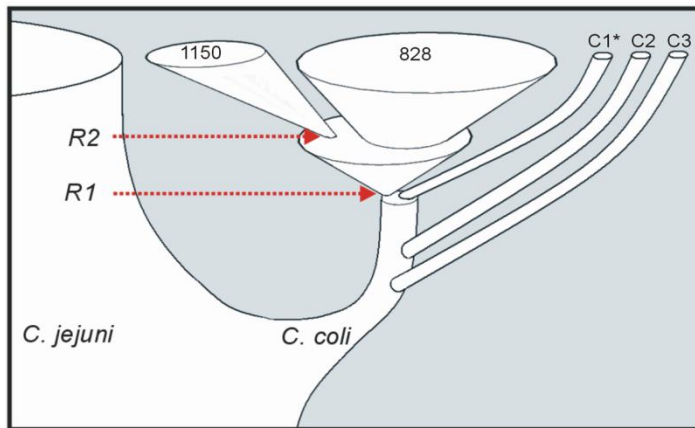
~ 400 genomes in NZ collection

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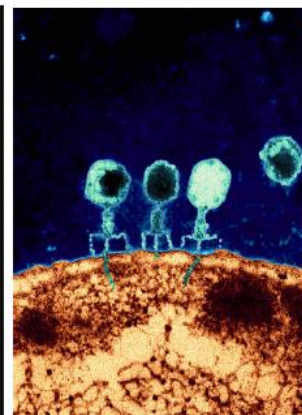# *Campylobacter:* Mechanisms of evolution

- NZs dominant enteric bacterial pathogen (8,000 cases)

- Mutation and recombination (HGT)

- Evidence from multiple studies indicate recombination <u>much </u>more important than mutation for *C. jejuni*

  - Natural transformation

  - Transduction

- See Tim Vaughan's talk
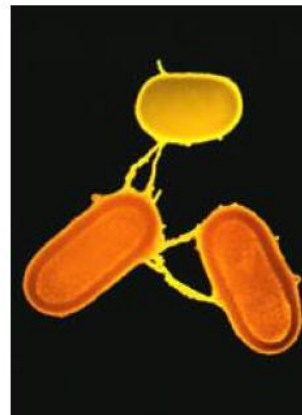
Conjugation   Transduction   Network of Life

© 2009, American Society for Microbiology
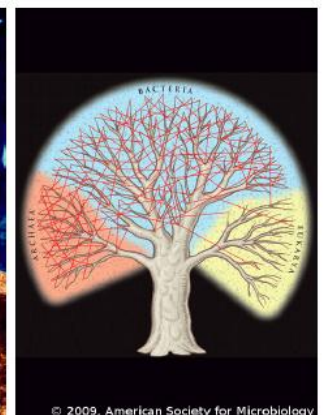
Estimating the Relative Roles of Recombination and Point Mutation in the Generation of Single Locus Variants in *Campylobacter jejuni* and *Campylobacter coli*

Shoukai Yu · Paul Fearnhead · Barbara R. Holland · Patrick Biggs · Martin Maiden · Nigel French

Analysis of Recombination in *Campylobacter jejuni* from MLST Population Data

Paul Fearnhead,[1] Nick G.C. Smith,[1] Mishele Barrigas,[2] Andrew Fox,[3] Nigel French[4]

ESTIMATING THE RELATIVE RATE OF RECOMBINATION TO MUTATION IN BACTERIA FROM SINGLE-LOCUS VARIANTS USING COMPOSITE LIKELIHOOD METHODS

BY PAUL FEARNHEAD* SHOUKAI YU[†] PATRICK BIGGS[†] BARBARA HOLLAND[‡] AND NIGEL FRENCH[†]

AOAS, resubmitted

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Ratio of recombination to mutation (events)

| Bacteria	Estimate of λ	95% CI
H. influenzae	4.9	(3.3,7.4)
S. aureus	1.4	(0.92,2.1)
S. uberis	11	(4.8,180)
C. jejuni	3.4	(2.9,4.1)
C. coli	0.43	(0.21,0.88)

TABLE 6

Estimate of common λ across MLST loci, together with putative 95% confidence intervals.

From Fearnhead et al 2014
Based on Single locus MLST variants – i.e. varied at one of 7 housekeeping genes

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PLoS one

Whole-Genome Comparison of Two *Campylobacter jejuni* Isolates of the Same Sequence Type Reveals Multiple Loci of Different Ancestral Lineage

Patrick J. Biggs[1]*, Paul Fearnhead[2], Grant Hotter[3], Vathsala Mohan[1], Julie Collins-Emerson[1], Errol Kwan[1], Thomas E. Besser[4], Adrian Cookson[5], Philip E. Carter[6], Nigel P. French[1]
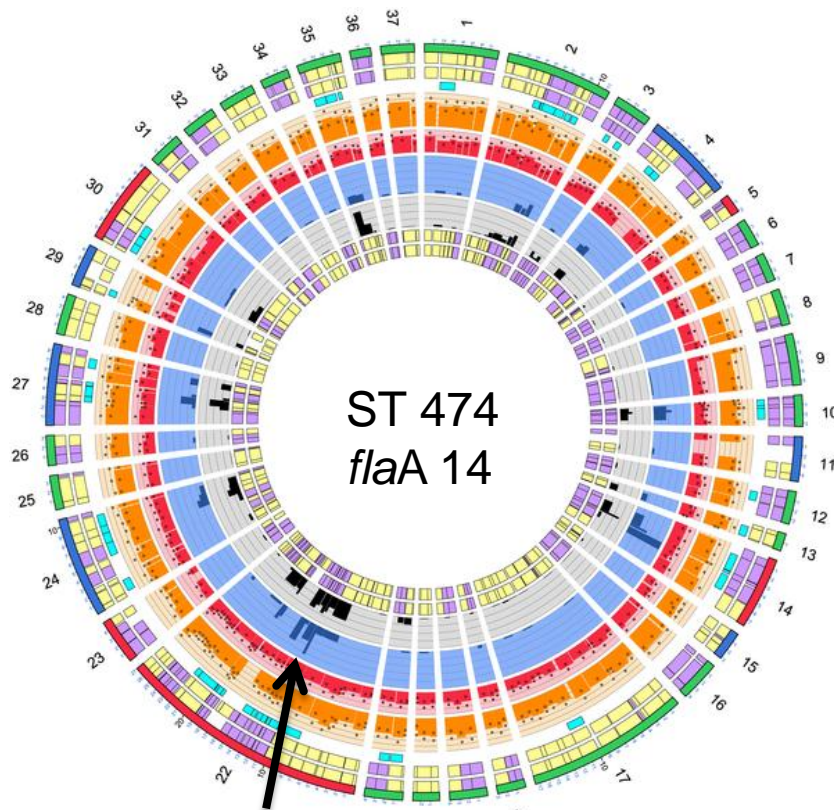

ST 474
*fla*A 14

Non-homologous recombination, *ykg*C

ST 474 evolution

- Compared just 2 isolates: Human and poultry isolates, same genotype, time and space.
- Epidemic strain
- 83 genes differed, 55 with amino acid differences.
- 96.7% were imported via recombination.
- Recombination much more important than mutation for generating divergence.
- Imported from multiple lineages since common ancestor
- MRCA ~ 120-130 years ago, recent population growth

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Host association and reservoir attribution
which animals are people getting infected from?
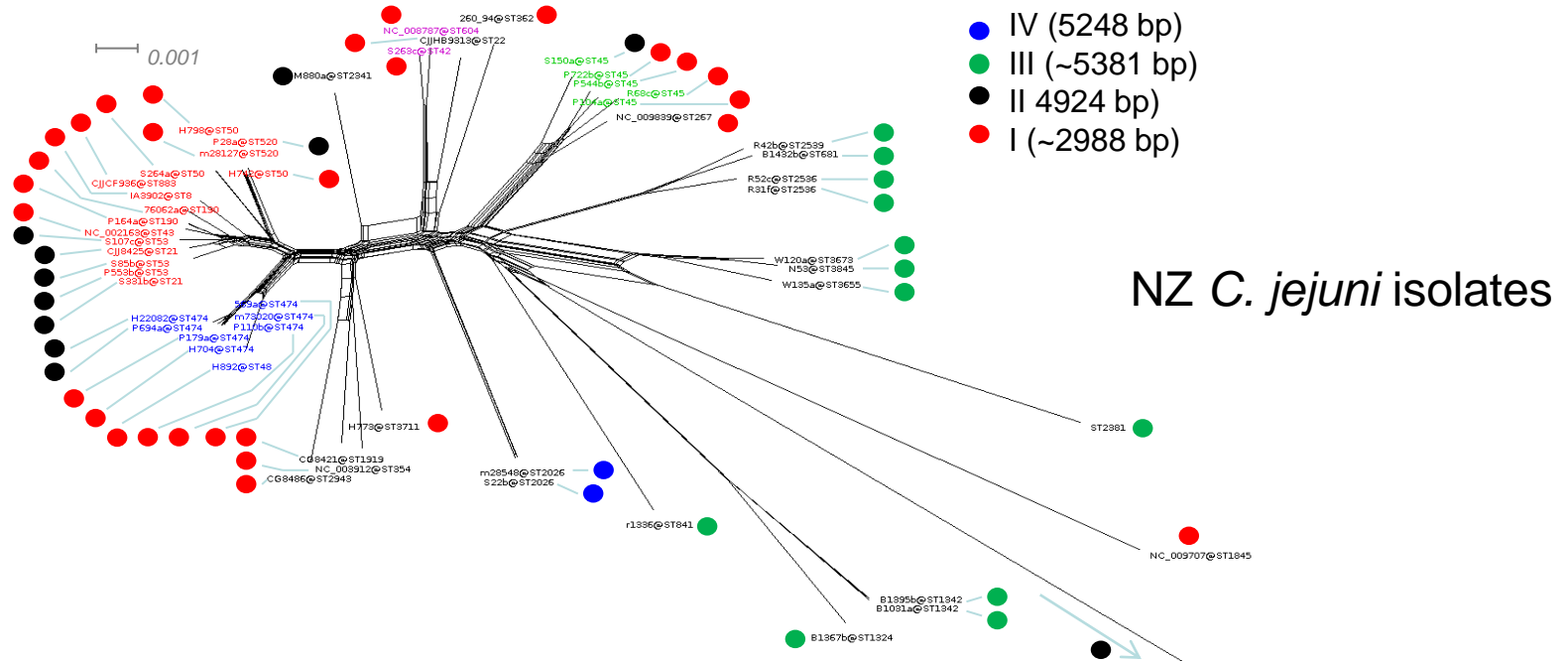
- Whole genome sequencing to identify markers associated with host
  - *ykg*C oxidoreductase – ruminant associated?
  - *Cj1069-rpsF* region
  - Sheppard et al vit B5 synthesis, GWAS study (*PNAS* 2013)

# Mutation and the short-term evolution of *C. jejuni in vivo* (Anja Friedrich)

- Chicken feed trial experiment
  - Caprylic acid
- Inoculated with *C. jejuni*
  - ST-474 and ST-45 strains
- Isolates recovered on days 33, 39 and 43
- 27 genome sequenced, + 2 parents

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Mutation and the short-term evolution of *C. jejuni in vivo* (Anja Friedrich)

- Only ST-474 recovered
  - 15 core SNPs/point mutations (kSNP*) confirmed
- 6 independent mutations in one gene
  - *mre*B, actin homologue involved in cell shape
  - No obvious cell shape changes?
- Other mutations in motility genes
  - *ptm*G flagellar glycosylation gene
  - Motility accessory factor
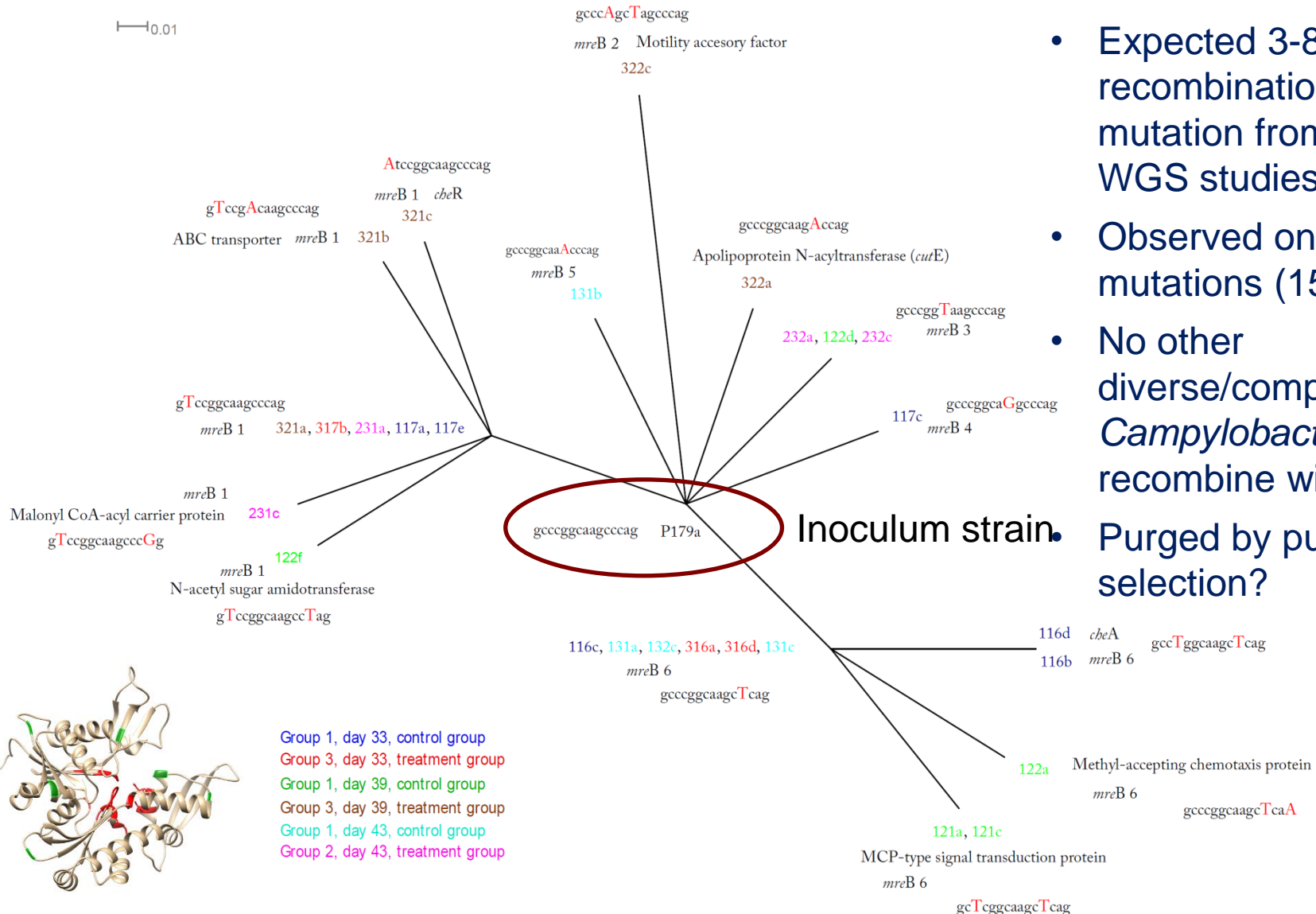
*Gardner and Hall 2013
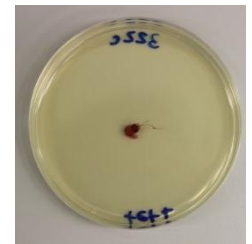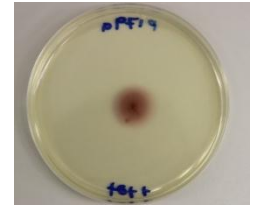
SEM of parent strain

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ki Pūrehu...
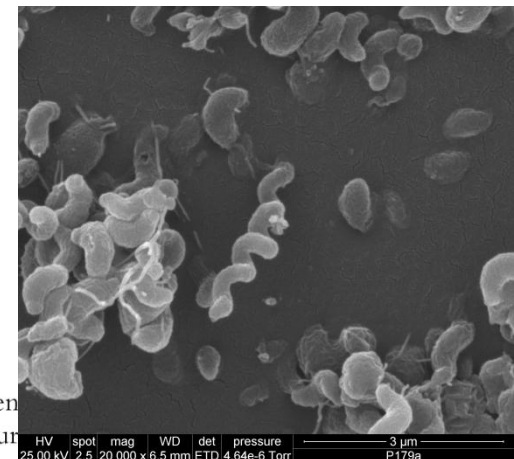
Multiple mutations in *mre*B

- No recombination?

- Expected 3-8:1 recombination to mutation from SLV and WGS studies

- Observed only mutations (15)

- No other diverse/compatible *Campylobacter* to recombine with?

- Purged by purifying selection?

Parallel evolution? (same SNPs in Oxford, UK human cases)

*mre*B 6

Chicken trial

Oxford cases

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Global view:
Campylobacter niche specialism

- Evidence of generalists and specialists
  - Agricultural strains
  - Wildlife strains

- *C. jejuni* just 4,000 years old? Traced back to common ancestor

- Species convergence / introgression
  - Associated with livestock strains
  - Sheppard et al *Science* 2008:
    Vol. 320 pp. 237-239

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Hybrid speciation in agricultural *Campylobacter*

C. coli lineages more common in fam and clinical sources

828 complex
19 21 24 15
5   2
16
23   17
1150 complex
10   • 25
1
11 12   • 18
            20
9 3
8 7 6

C. coli lineages more
common in riparian sources

29
22   28
30 13 26 4 27

C. jejuni

Sheppard et al 2011

Progressive genome-wide introgression in agricultural *Campylobacter coli*

- Hybridization facilitates niche adaptation in diverse taxa.
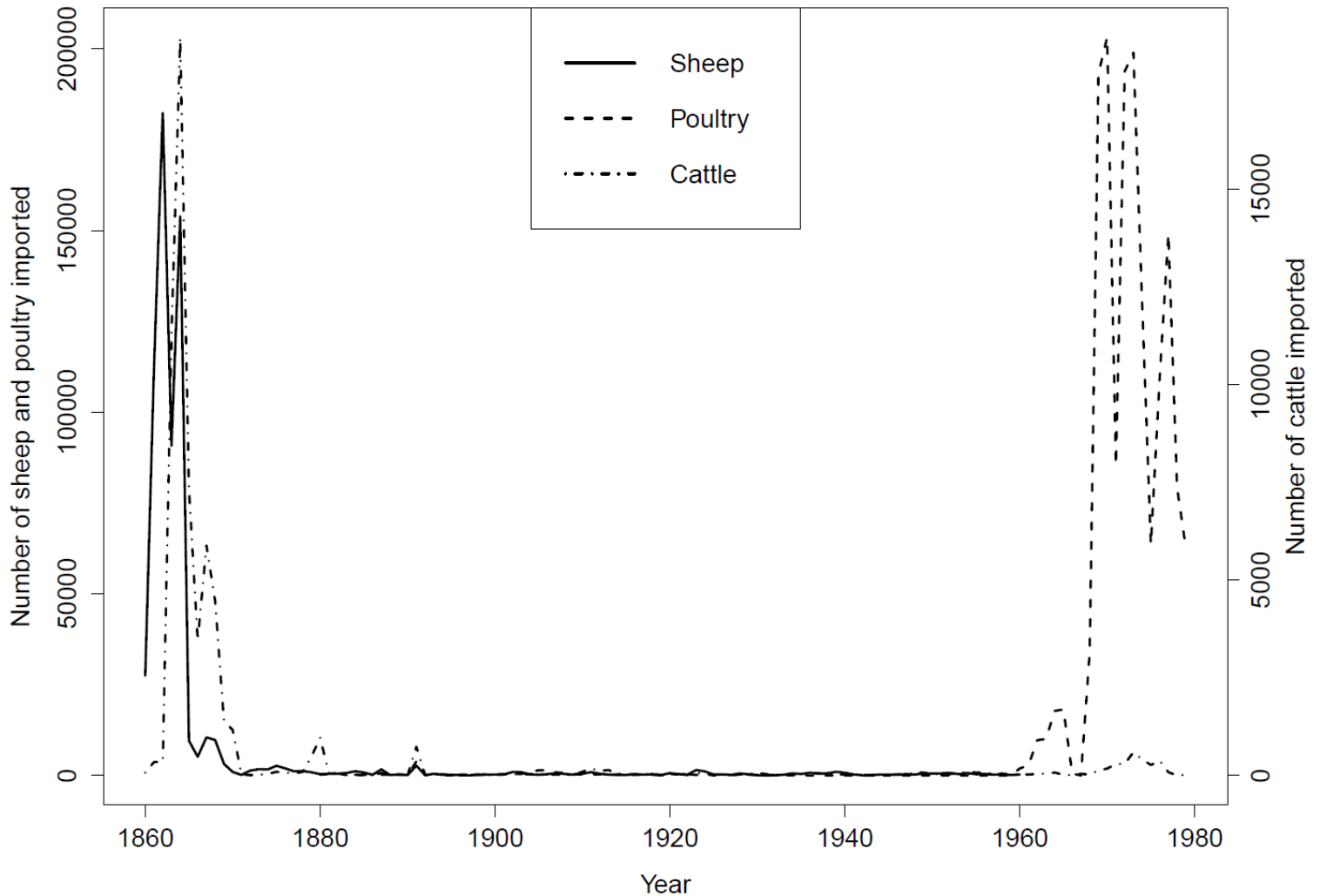
- Introgression mainly *jejuni - coli*

1150   828   C1* C2 C3

R2

R1

C. jejuni   C. coli
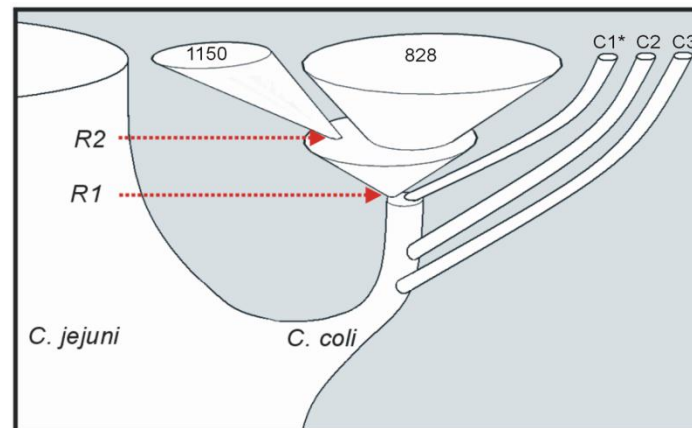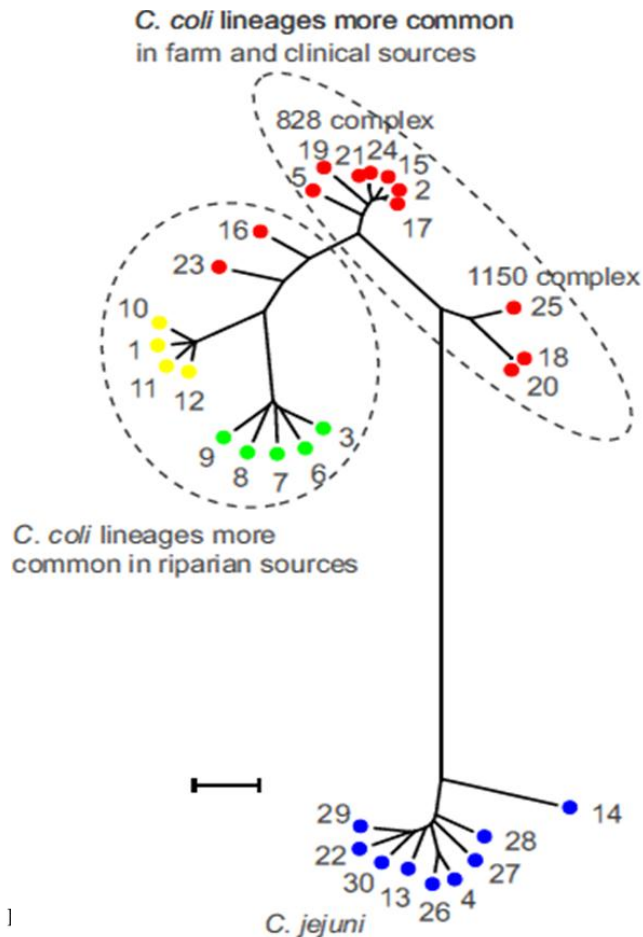
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Livestock populations in NZ

Barbara Binney

Binney et al, New Zealand Vet Journal 2014

Tracing genealogical histories…

- Epidemic *Campylobacter jeuni* strain ST 474 - common ancestor introduced in ruminants early 1800s?

- Bottleneck and subsequent expansion as poultry industry expanded?

Evolution of *Campylobacter* Species in New Zealand

17

Nigel French, Shoukai Yu, Patrick Biggs, Barbara Holland, Paul Fearnhead, Barbara Binney, Andrew Fox, Dai Grove-White, Jessica W. Leigh, William Miller, Petra Muellner and Philip Carter

In *Campylobacter Ecology and Evolution.* Eds Sheppard, Méric

Compatibility analysis
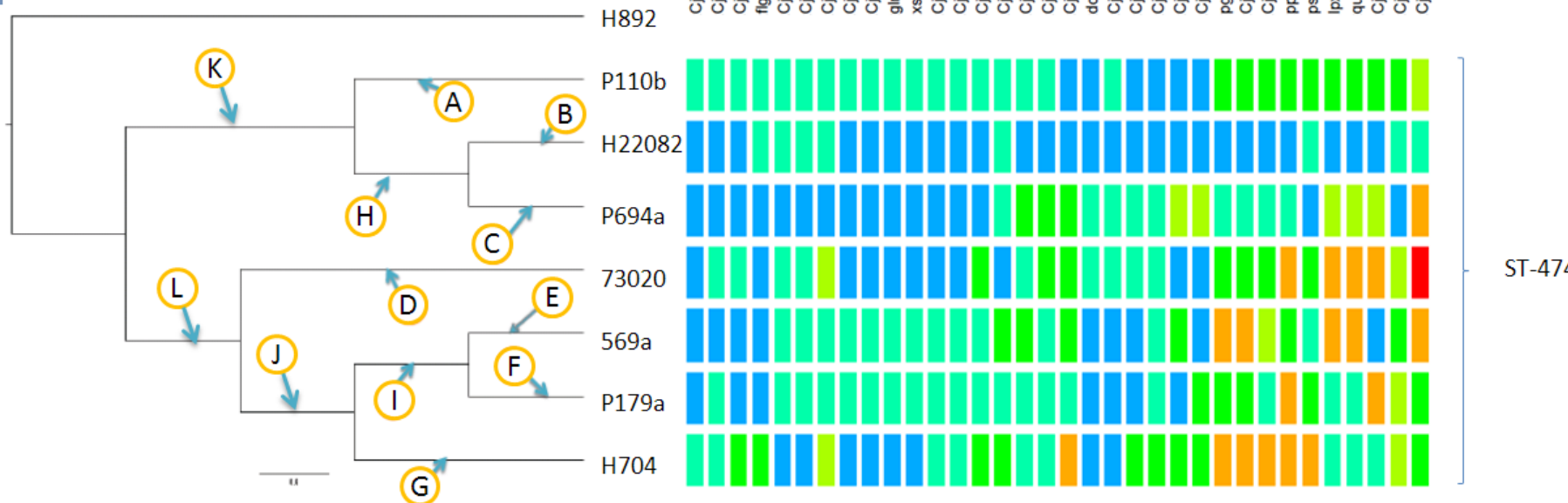
Novel Clonal Complexes with an Unknown Animal Reservoir Dominate *Campylobacter jejuni* Isolates from River Water in New Zealand[▽]

P. E. Carter,[1]* S. M. McTavish,[1]† H. J. L. Brooks,[2] D. Campbell,[3] J. M. Collins-Emerson,[4]
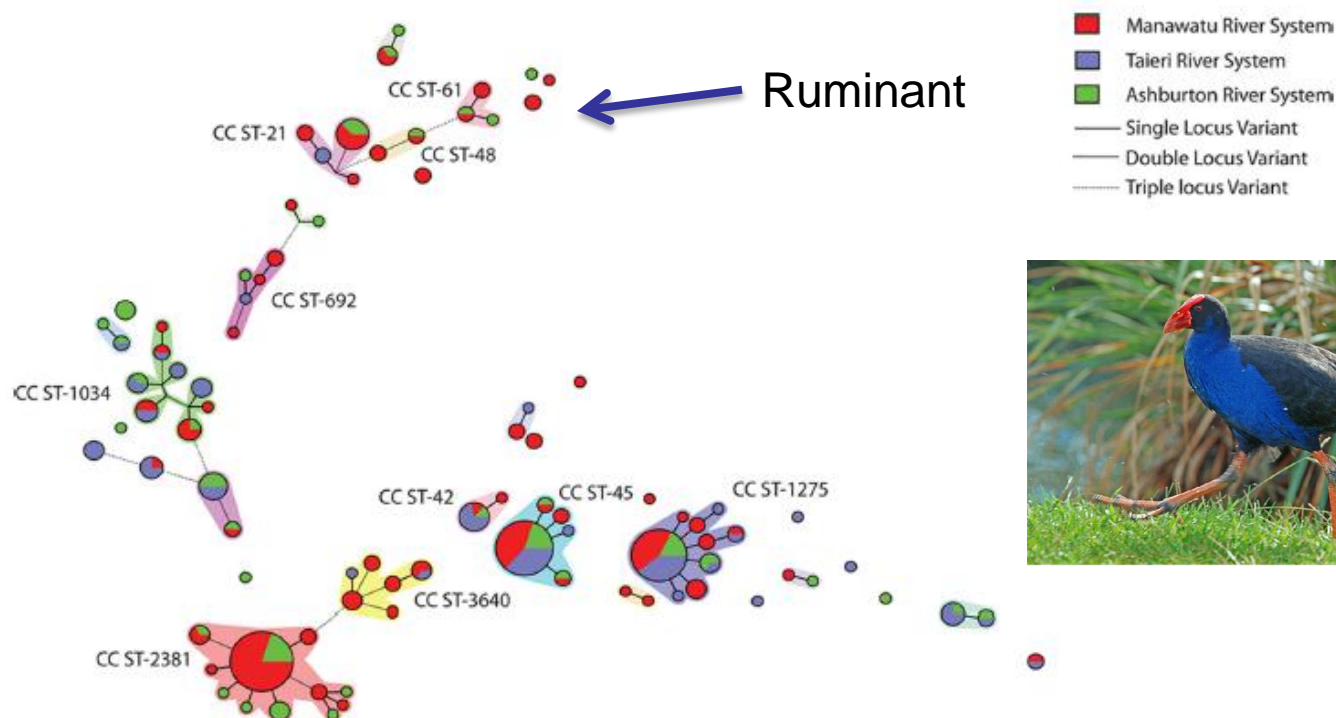A. C. Midwinter,[4] and N. P. French[4]

Ruminant

FIG. 1. Minimum spanning tree of New Zealand water isolates based on sequence types. Clusters are identified by the clonal complex number sociated with the sequence types in the cluster.

Campylobacter jejuni sources in water

Most Campylobacter in water in Manawatu from wildlife – even in dairy catchments

Water birds

Sheep

Cattle

Key
1. Ororua
2. Himatangi
3. Mangapapa
4. Manawatu A
5. Tokomaru
6. Manawatu H

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Pathogen prediction (Barbara Binney)

Whole genome sequencing ~ 400 NZ isolates

	MLST	Pathogenic Families Matched	prediction score	predicted to be human pathogen	
W194b	ST2381	40	79.701	yes	Water rail associated
N3d	ST2381	41	84.042	yes	Water rail associated
W83a	ST2381	44	97.693	yes	Water rail associated
N191	ST42	428	858.871	yes	Cattle associated
S263a	ST42	430	865.367	yes	Cattle associated
N31	ST42	442	889.374	yes	Cattle associated

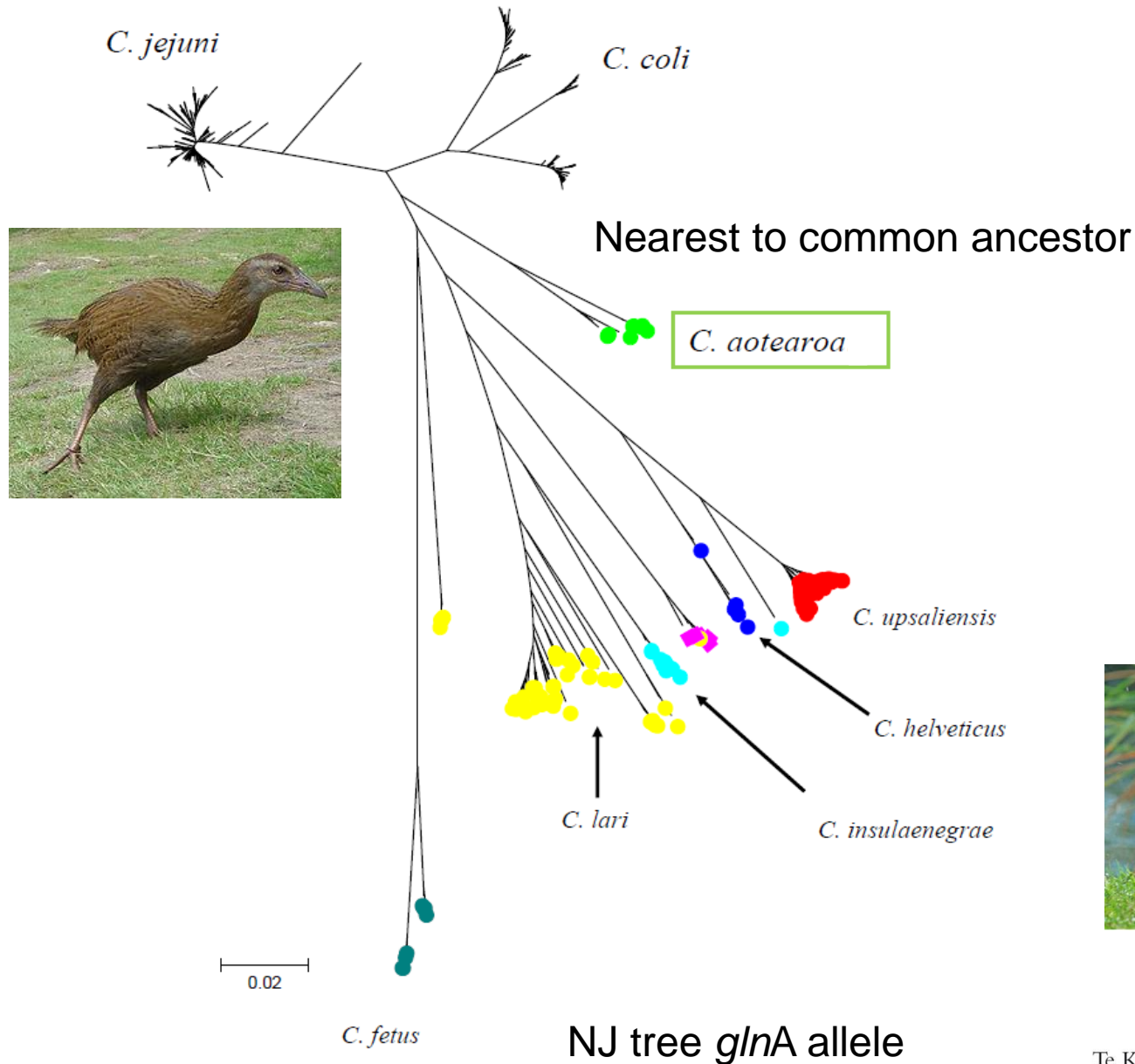Some strains of *C. jejuni* less pathogenic? Consistent with epidemiology

PathogenFinder - Distinguishing Friend from Foe Using Bacterial Whole Genome Sequence Data

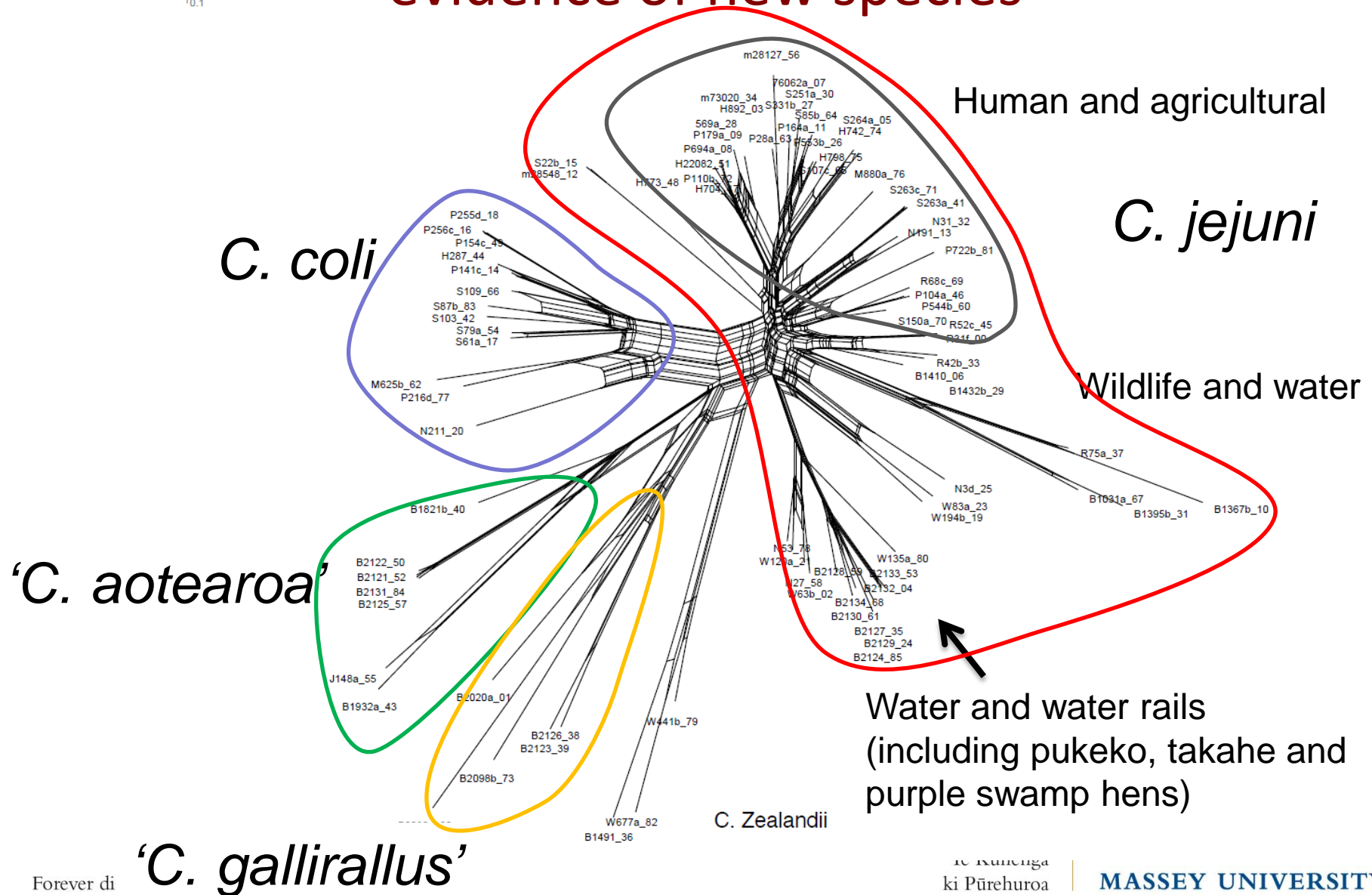Salvatore Cosentino[1]*, Mette Voldby Larsen[1], Frank Møller Aarestrup[2], Ole Lund[1]

1 Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark, 2 National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark

Multiple water rail associated lineages in New Zealand

Nearest to common ancestor

C. jejuni

C. coli

C. aotearoa

C. upsaliensis

C. helveticus

C. lari

C. insulaenegrae

C. fetus

0.02

NJ tree *gln*A allele

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Pan-genome analysis NZ *Campylobacter*: evidence of new species

Human and agricultural

C. jejuni

C. coli

Wildlife and water

'*C. aotearoa*'

'*C. gallirallus*'

C. Zealandii

Water and water rails (including pukeko, takahe and purple swamp hens)
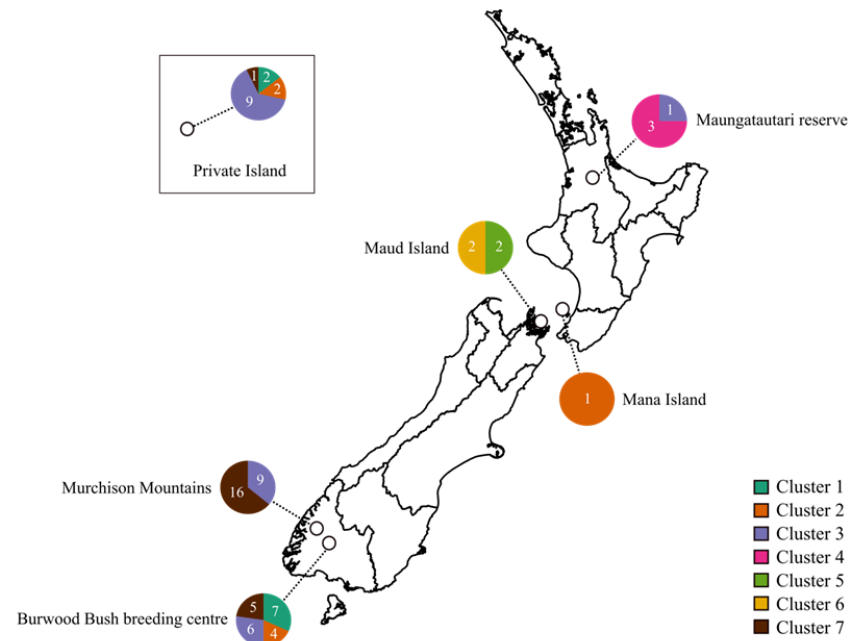
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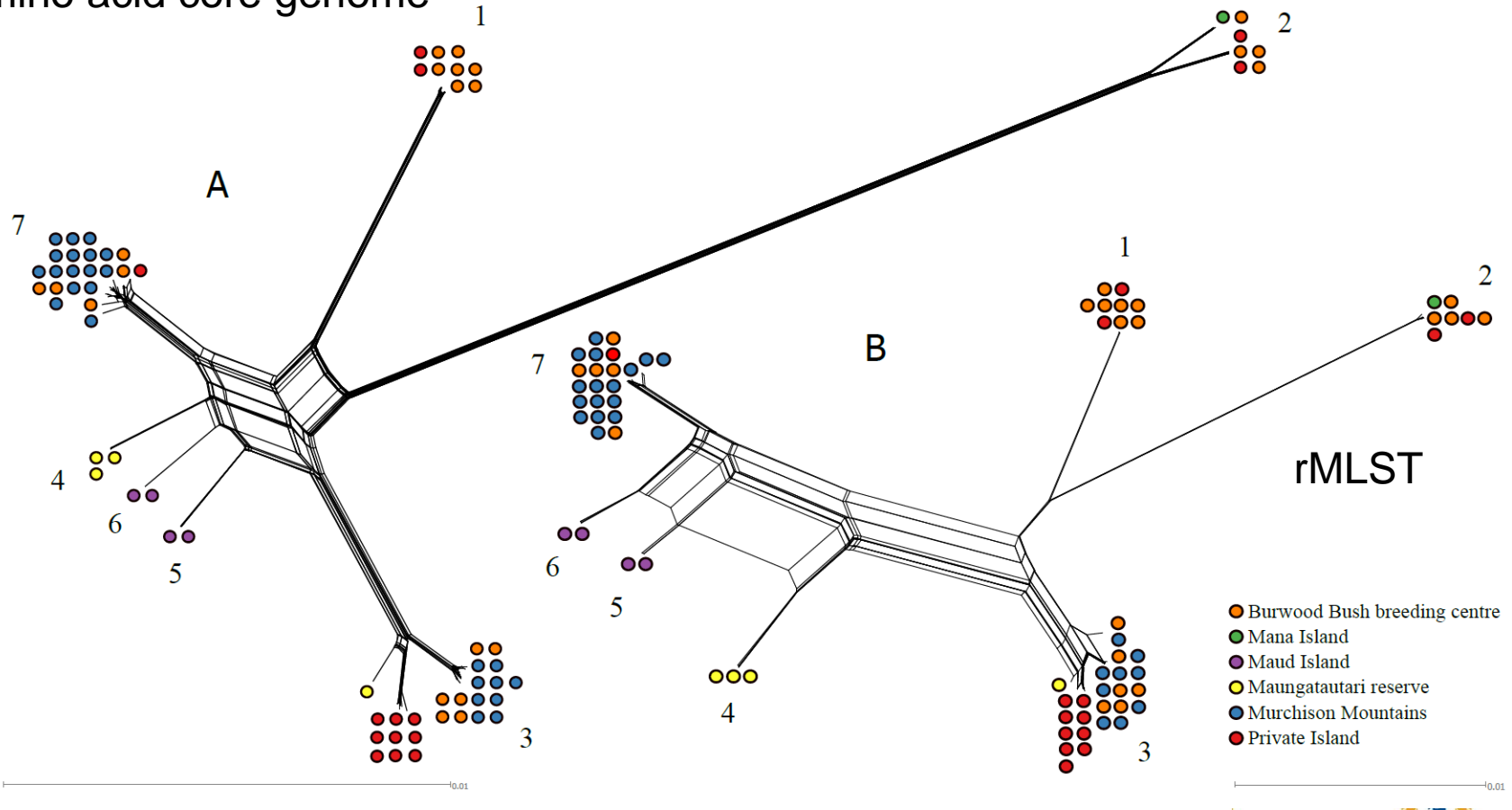ki Pūrehuroa | MASSEY UNIVERSITY

Takahē *Campylobacter* study

- Aim: Investigate the influence of geographic isolation and translocation on the molecular differentiation of commensal *Campylobacter*

- Faecal samples and swabs 118 takahē
  - Nov 2011 - Apr 2013

- 6 locations in NZ

Map of New Zealand showing pie charts at sampling locations: Private Island, Maungatautari reserve, Maud Island, Mana Island, Murchison Mountains, Burwood Bush breeding centre.

Legend:
- Cluster 1
- Cluster 2
- Cluster 3
- Cluster 4
- Cluster 5
- Cluster 6
- Cluster 7

C. Aotearoa 70 genome*:*
location and population structure

Amino acid core genome

rMLST

Burwood Bush breeding centre
Mana Island
Maud Island
Maungatautari reserve
Murchison Mountains
Private Island

Strong evidence of spatial structuring of genotypes according to translocation site
Implications for translocation as a conservation tool

Salmonellosis: multiple serotypes, complex epidemiology

- In NZ salmonellosis is the second most notified foodborne disease after campylobacteriosis.

Table 3.1: Total number of cases from January 2000 to December 2009 for the ten most prevalent serotypes

Serotype	Number of Cases
S. Typhimurium DT160	2592
S. Typhimurium DT1	1010
S. Typhimurium DT135	844
S. Brandenburg	734
S. Typhimurium DT156	705
S. Infantis	657
S. Typhimurium DT101	570
S. Enteritidis phage type 9a	544
S. Typhimurium DT42	334
S. Saint Paul	310

Non-typhoidal >15,000 cases

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Typhimurium DT 160 most prevalent serotype until 2011

- In 1998, *Salmonella* DT160 identified as a human pathogen in NZ

- Initial outbreak in Christchurch in humans and sparrows (2000)

- Subsequently isolated from farmed animals and wild birds.

- After 2000, increased significantly progressing from the South to the North Island.

- Now 2nd behind Typhimurium RDNC-May 06.

R.E. Barbash Photography

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DT160 epidemiology

The evolution and distribution of phage ST160 within *Salmonella enterica* serotype Typhimurium

M. Price-Carter and others

Epidemiology and Infection, 139(8):1262-71, 2011
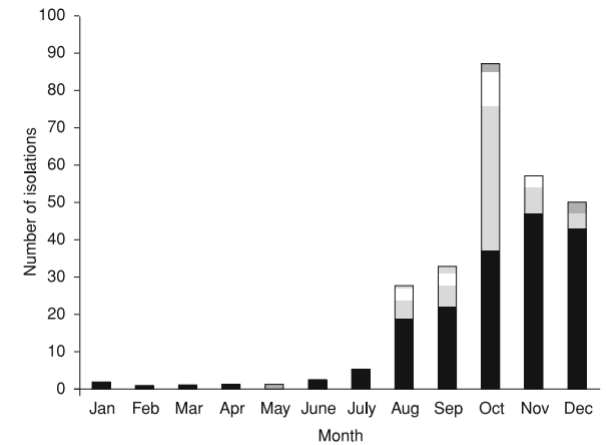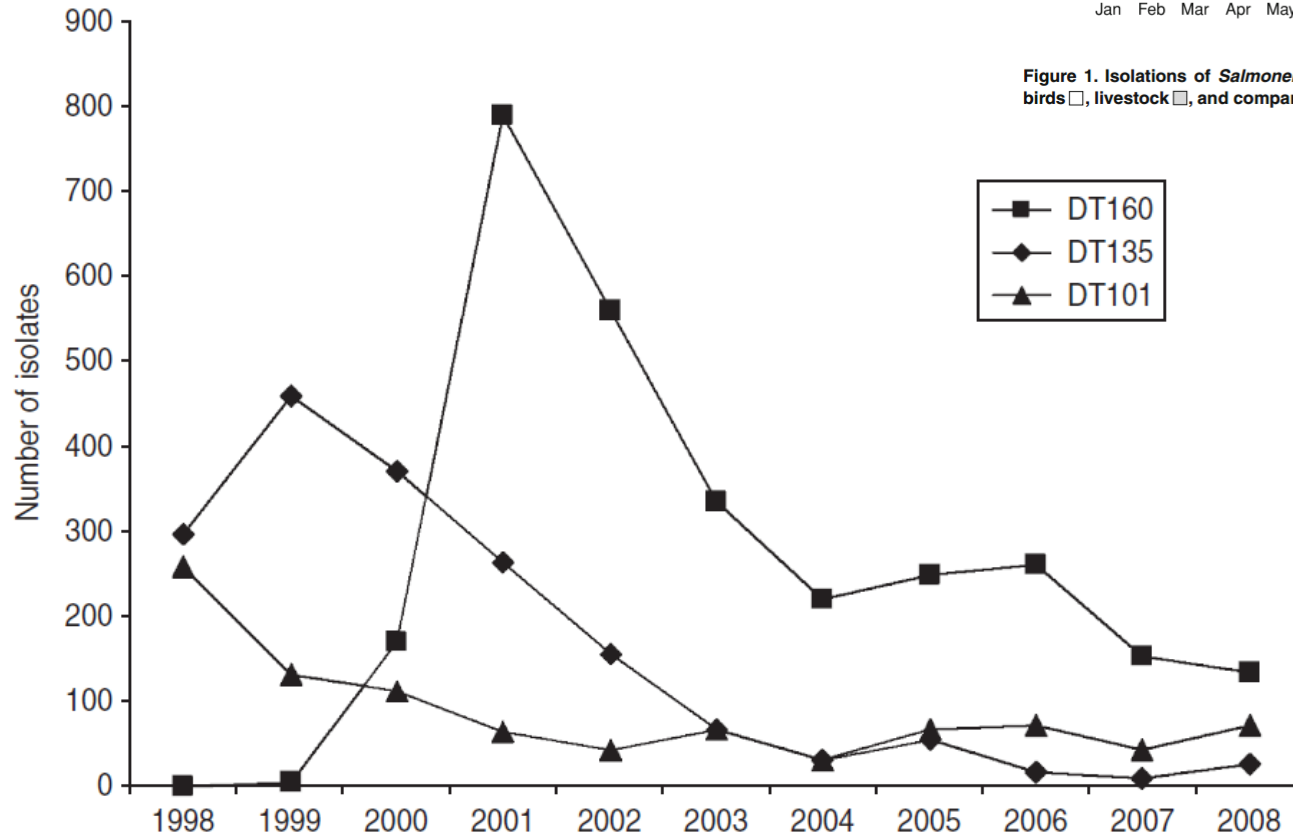
Figure 1. Isolations of *Salmonella* Typhimurium DT160 in humans ■, birds □, livestock □, and companion animals ▨ during 2000.

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Cases2kml (Chris Jewell)

Salmonellosis in
humans 2000-2009

Blue=Brandenburg
(sheep, direct contact)

Purple=Typhimurium 156
(food, cattle)

Green=Typhimurium 160
(wild birds, food)

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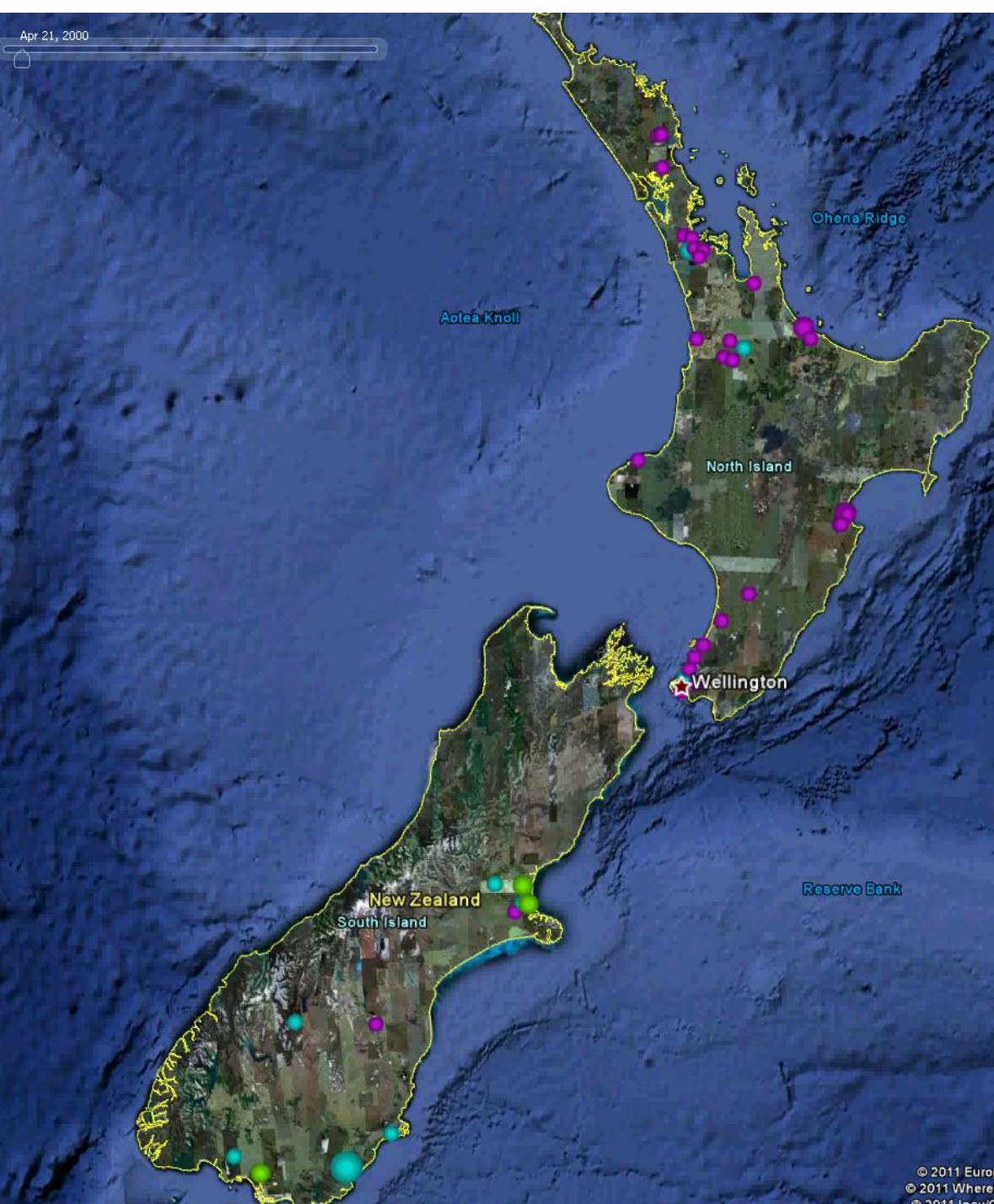
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Salmonellosis in
humans 2000-2009

Blue=Brandenburg
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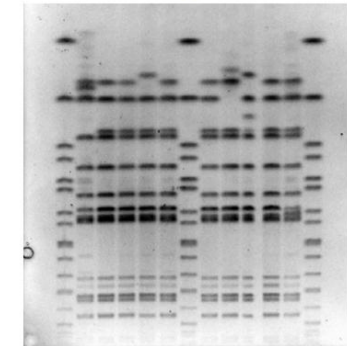
S. Tyhimurium DT 160: initial genotyping study

Omar et al 2010

UPGMA Dendrogram based on *Spe*I	PFGE *Spe*I	PFGE *Xba*I	PCR based virulotyping pefA	sifA	sopE	invA	iroN	*Spe*I *Xba*I		Vir	Lab ID	Source	Date isolated
								A	D	1	ERL06/2065	Human Otago	2006-06-01
								A	A*	1	ERL023275	Avian Cockatoo	2002-08-01
								A	A	2	ERL032131	Human	2003-05-01
								A	A	2	ERL072182	Avian Sparrow	2007-07-01
								A	B	1	ER0103668	Human Auckland	2001-08-01
								A	A*	1	ER0104613	Avian White eye	2001-09-01
								A	A	1	ER0100739	Human	2001-02-01
								A	A	3	ERL03695	Avian Sparrow	2003-02-01
								A	A	1	Common type: multiple sources **		
								A	E	1	ERL053850	Human	2005-10-01
								A	A	2	ER0004663	Avian Sparrow	2000-10-01
								A	A	5	ERL083326	Human	2008-09-01
								A	G	1	F98	Reference	
								A	F	6	SL1344	Reference	
								B	C	4	ER0003038	Avian Sparrow	2000-08-01
								C	A	1	ERL091904	Human	2009-06-01
								D	A	1	ERL02/1077	Poultry Environmental	2002-03-01
								E	A	1	ERL042607	Human	2004-08-01

**Total of 76 isolates comprising 23 human, 24 avian, 29 poultry environment.
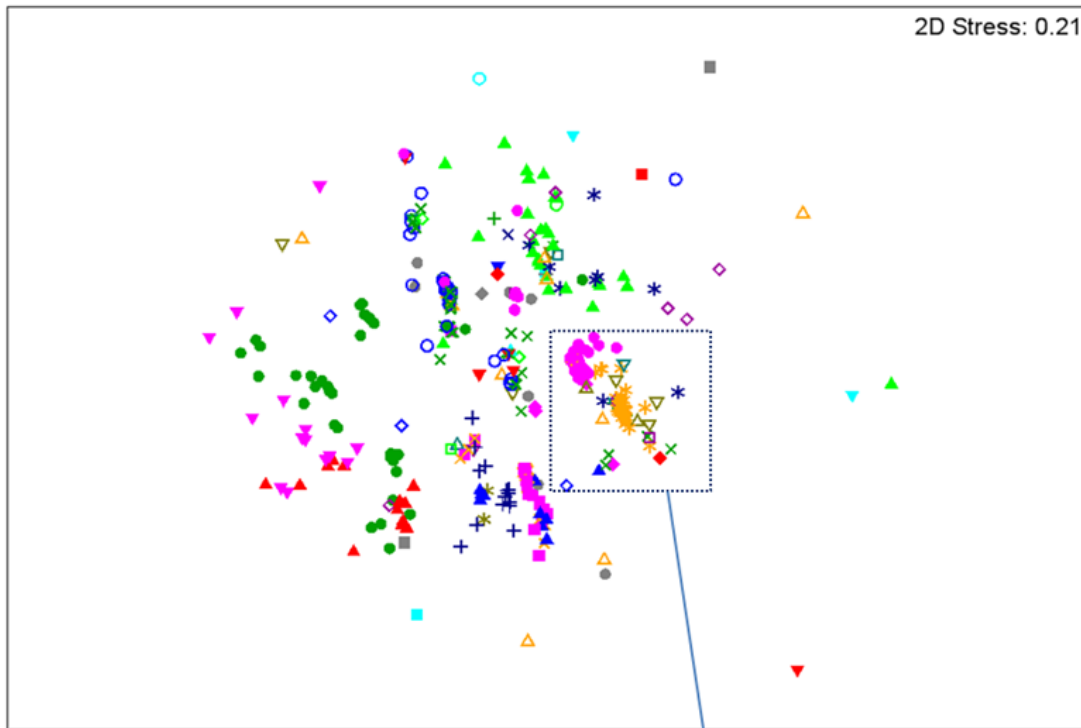
Pleydell et al 2013

Multi-dimensional scaling plot of PFGE distance matrix: *Salmonella* Typhimurium

DT160 evolved into RDNC-May 06?

Genome sequencing: preliminary SNP analysis
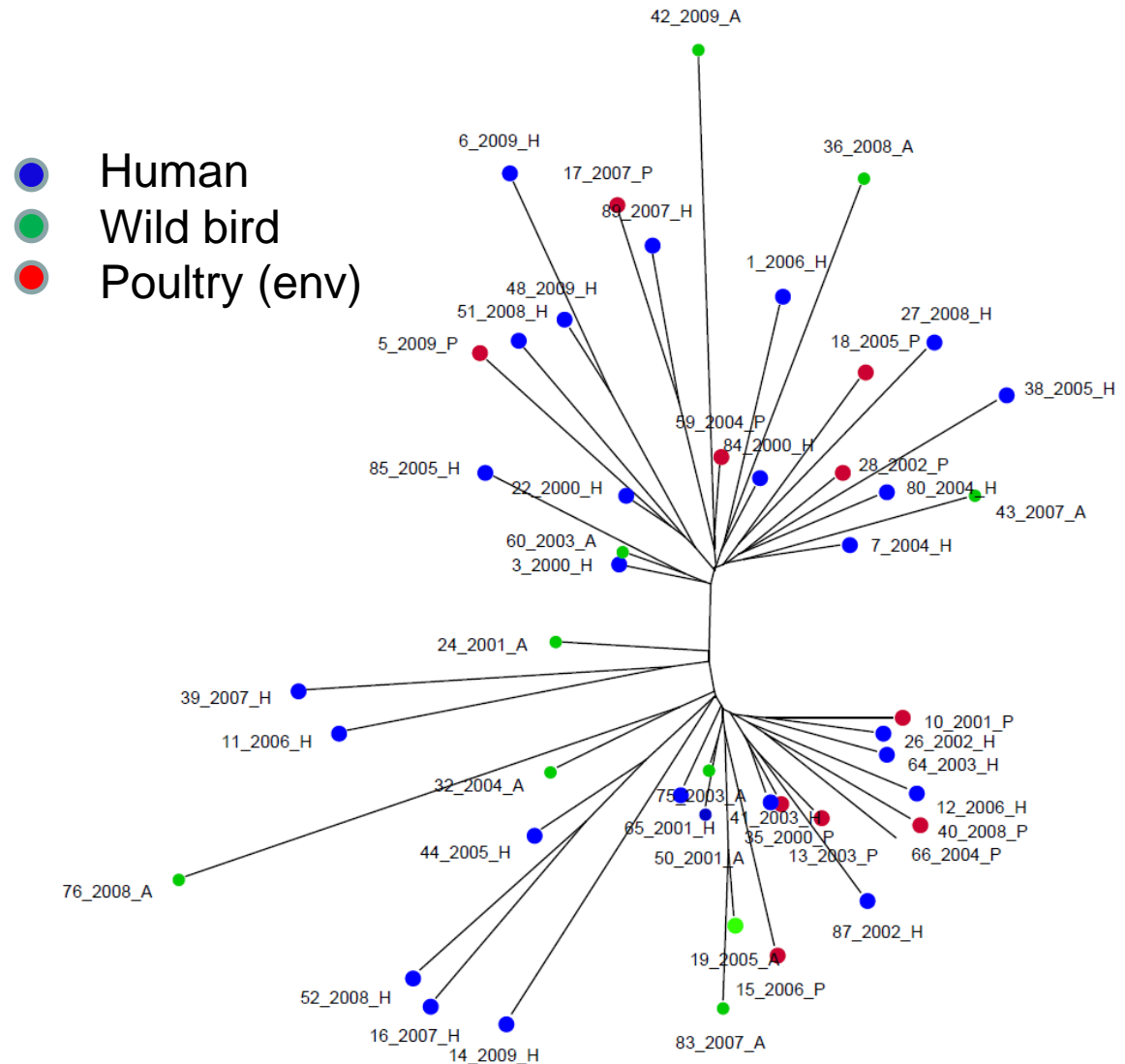
Sam Bloomfield

50/120 isolates sequenced

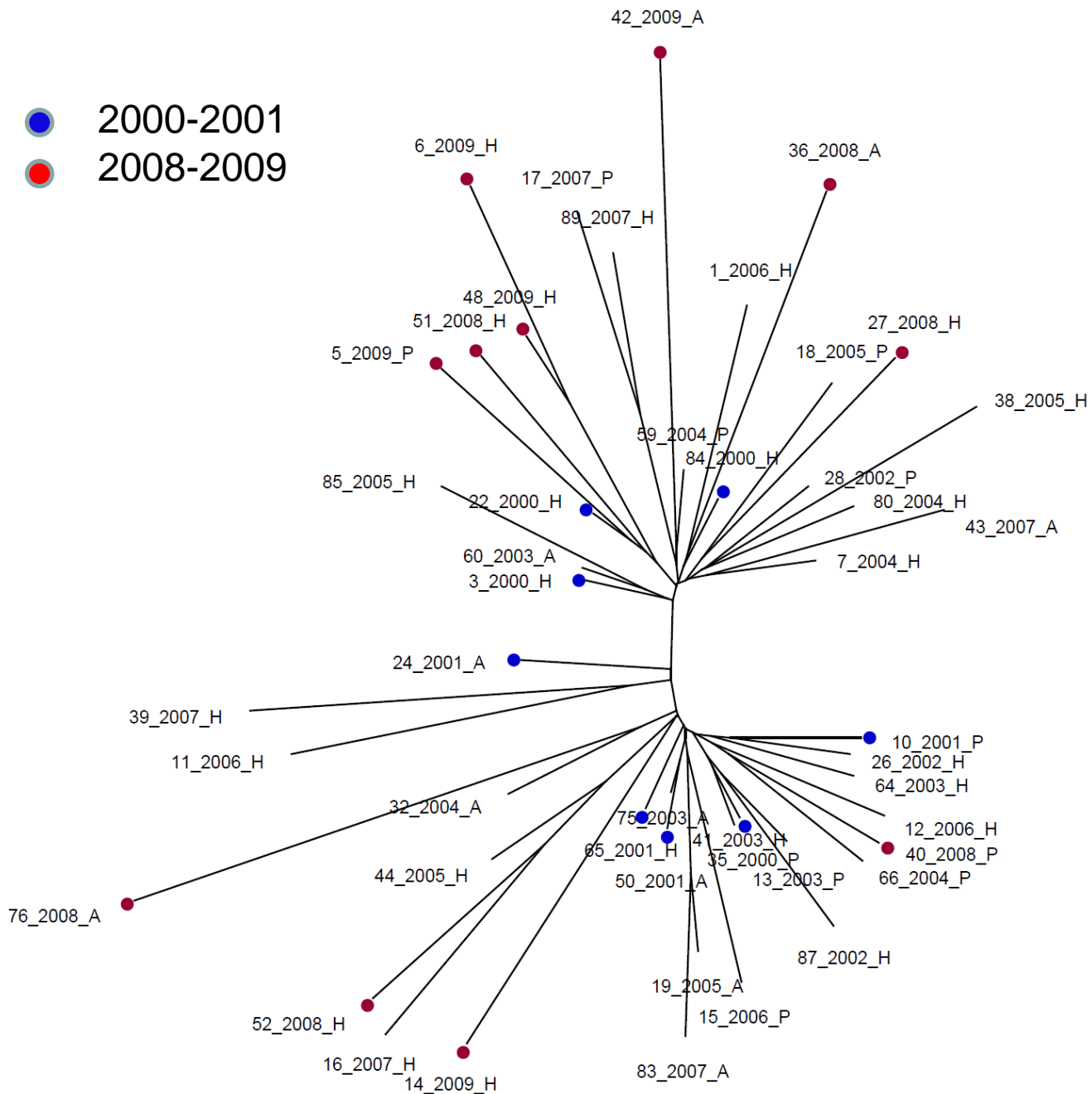~400 SNPs (needs full QA)

Resolves phylogeny – all unique profiles
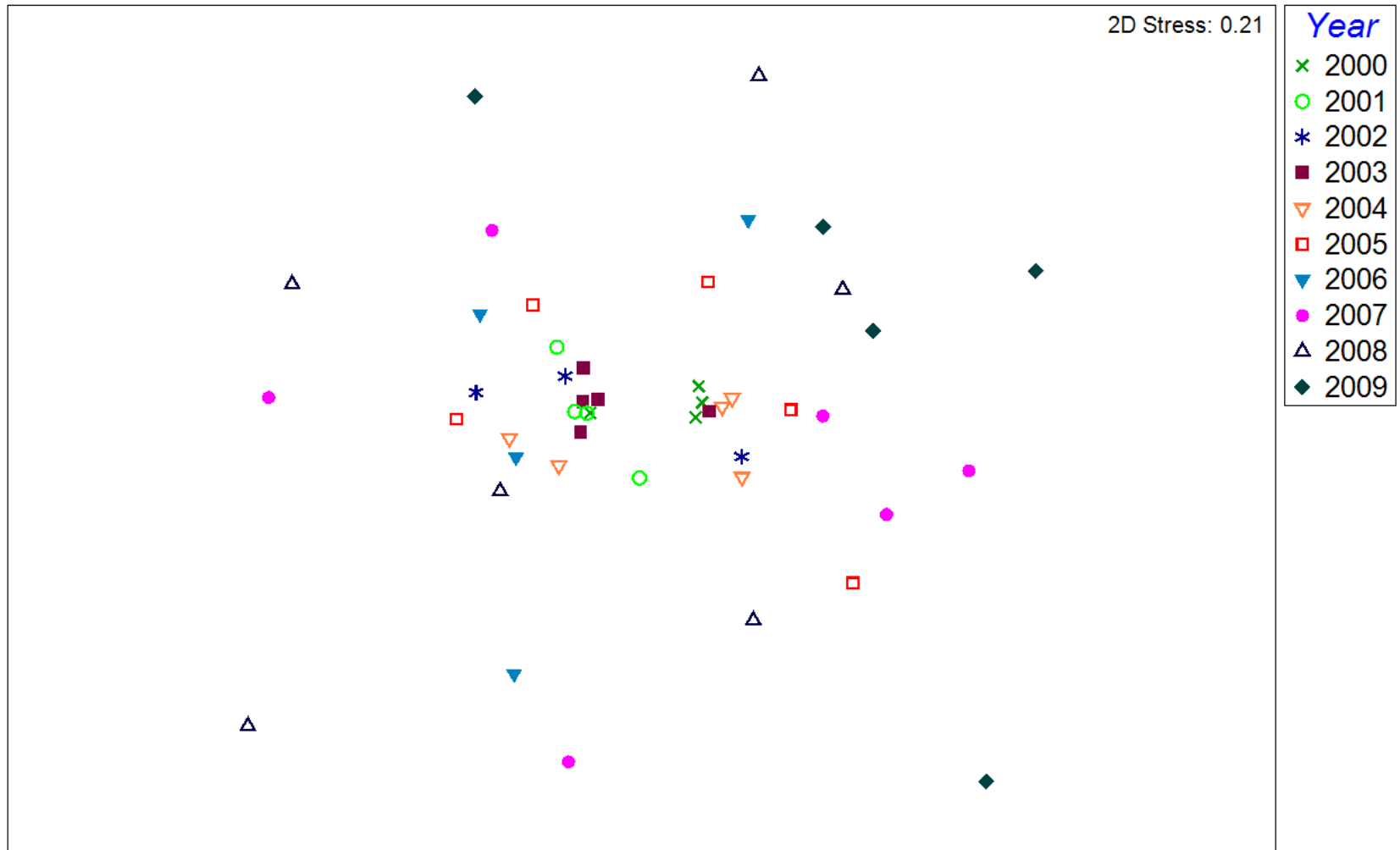
Evidence for zoonotic spread
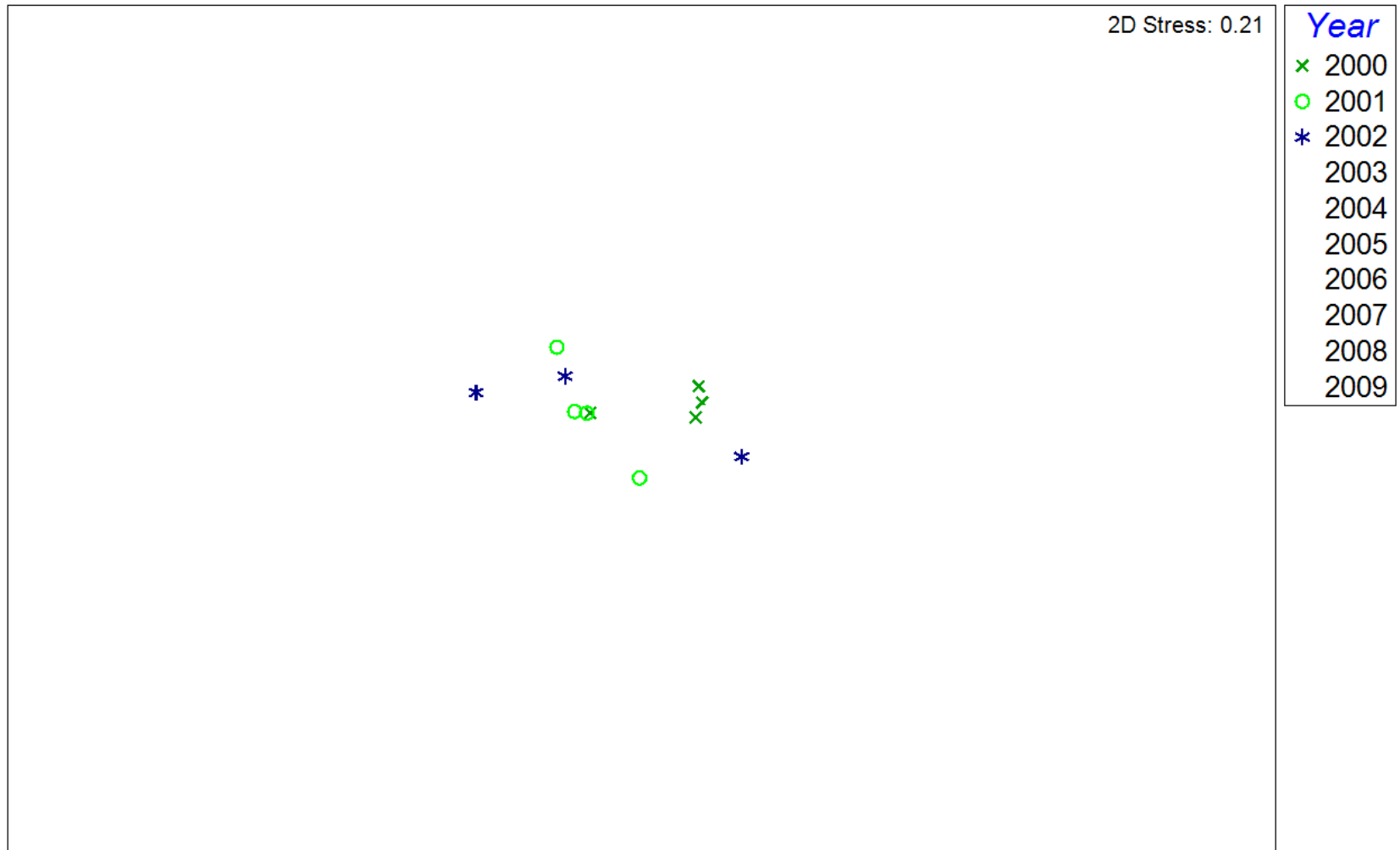
Collaboration with ESR: Phil Carter, Muriel Dufour

More recent isolates peripheral in tree

2000-2001
2008-2009

42_2009_A
6_2009_H
17_2007_P
89_2007_H
36_2008_A
1_2006_H
48_2009_H
51_2008_H
27_2008_H
5_2009_P
18_2005_P
38_2005_H
59_2004_P
84_2000_H
28_2002_P
85_2005_H
80_2004_H
22_2000_H
43_2007_A
60_2003_A
7_2004_H
3_2000_H
24_2001_A
39_2007_H
10_2001_P
11_2006_H
26_2002_H
64_2003_H
32_2004_A
75_2003_A
12_2006_H
65_2001_H
41_2003_H
40_2008_P
44_2005_H
35_2000_P
66_2004_P
50_2001_A
13_2003_P
76_2008_A
87_2002_H
19_2005_A
52_2008_H
15_2006_P
16_2007_H
14_2009_H
83_2007_A

Mutidimensional scaling based on core genome SNPs

2D Stress: 0.21

Year
× 2000
○ 2001
✳ 2002
■ 2003
▽ 2004
□ 2005
▼ 2006
● 2007
△ 2008
◆ 2009

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Mutidimensional scaling based on core genome SNPs

Mutidimensional scaling based on core genome SNPs
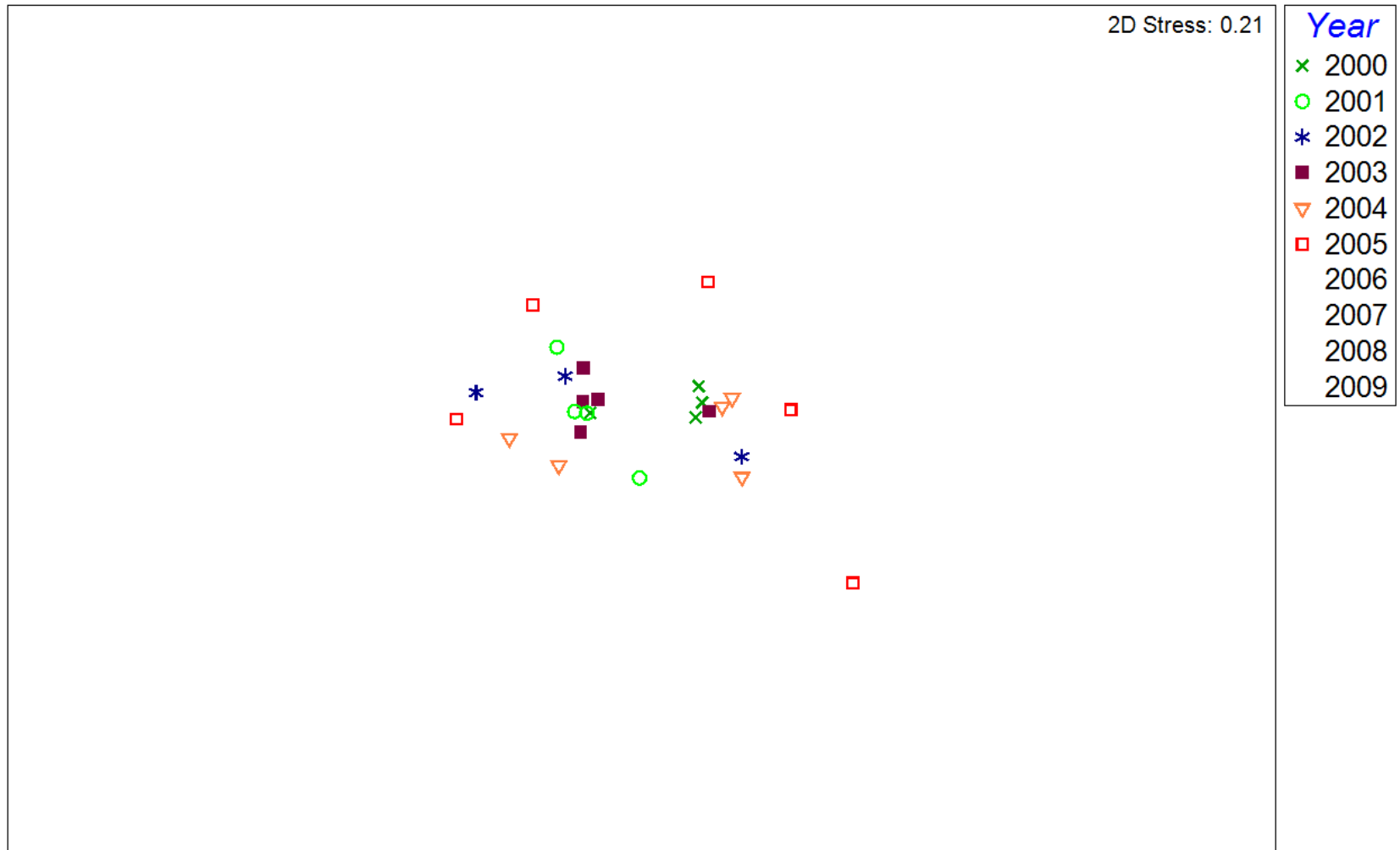
# Mutidimensional scaling based on core genome SNPs
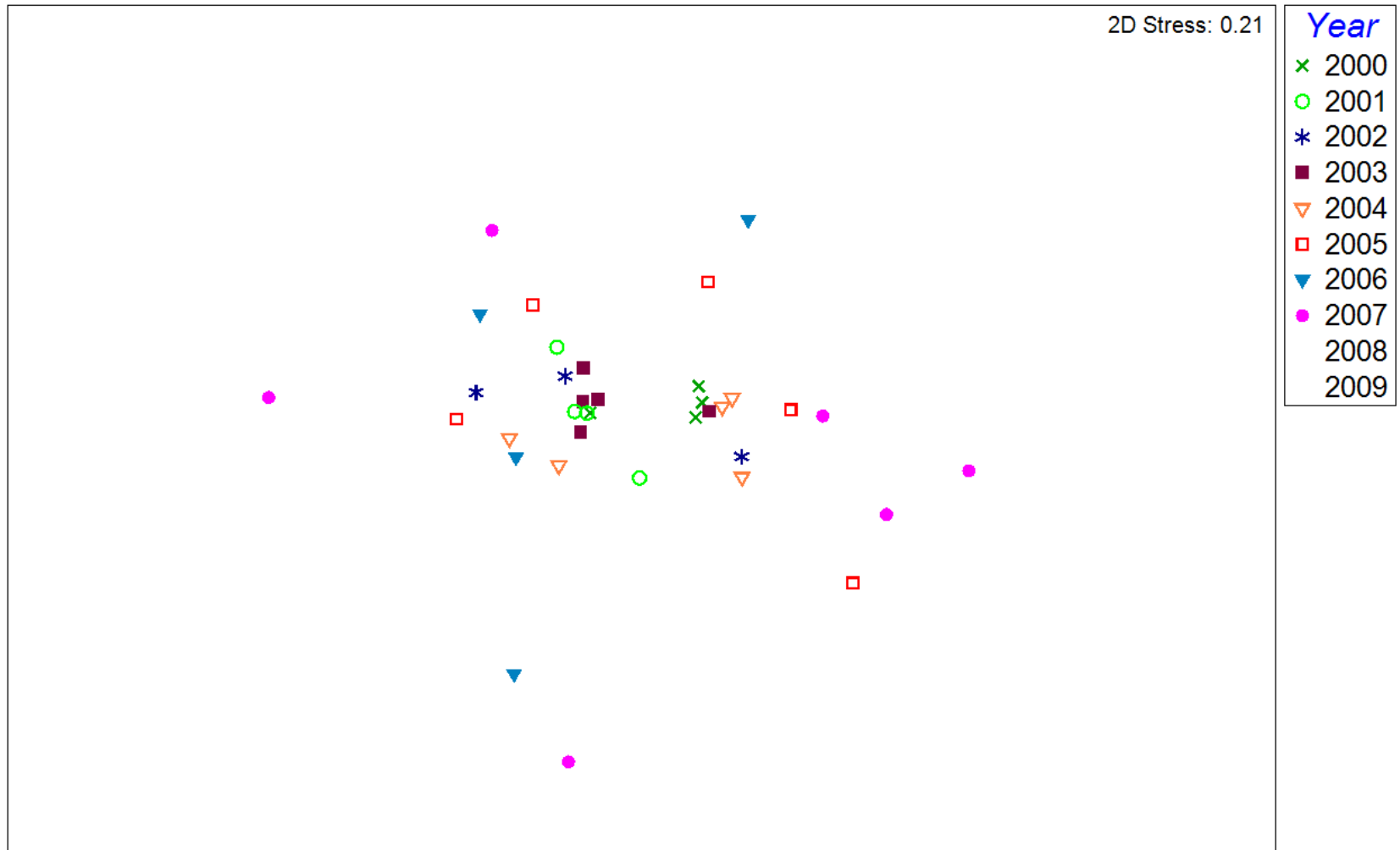
Mutidimensional scaling based on core genome SNPs
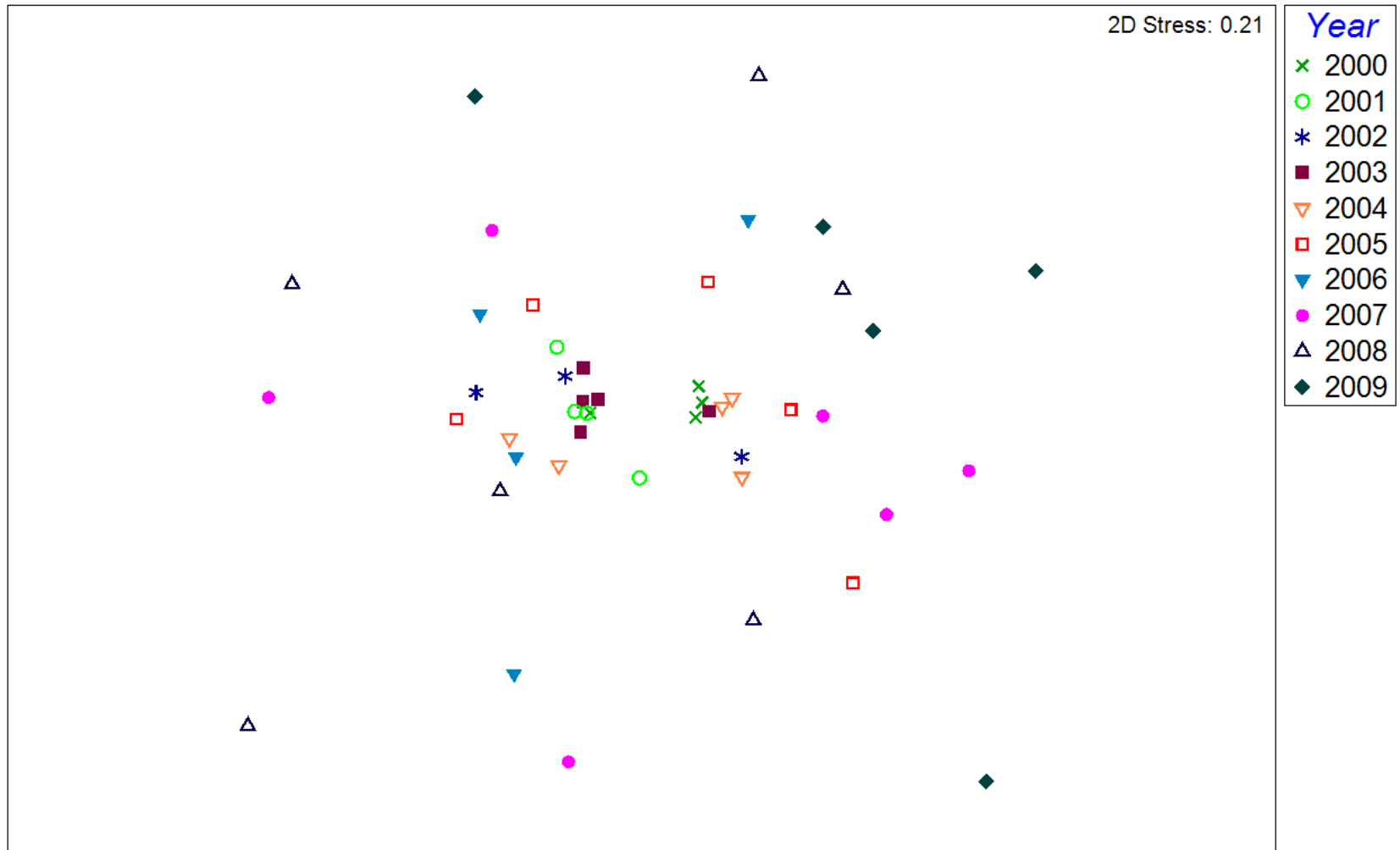
Relationship between year of 'epidemic' and genetic dissimilarity
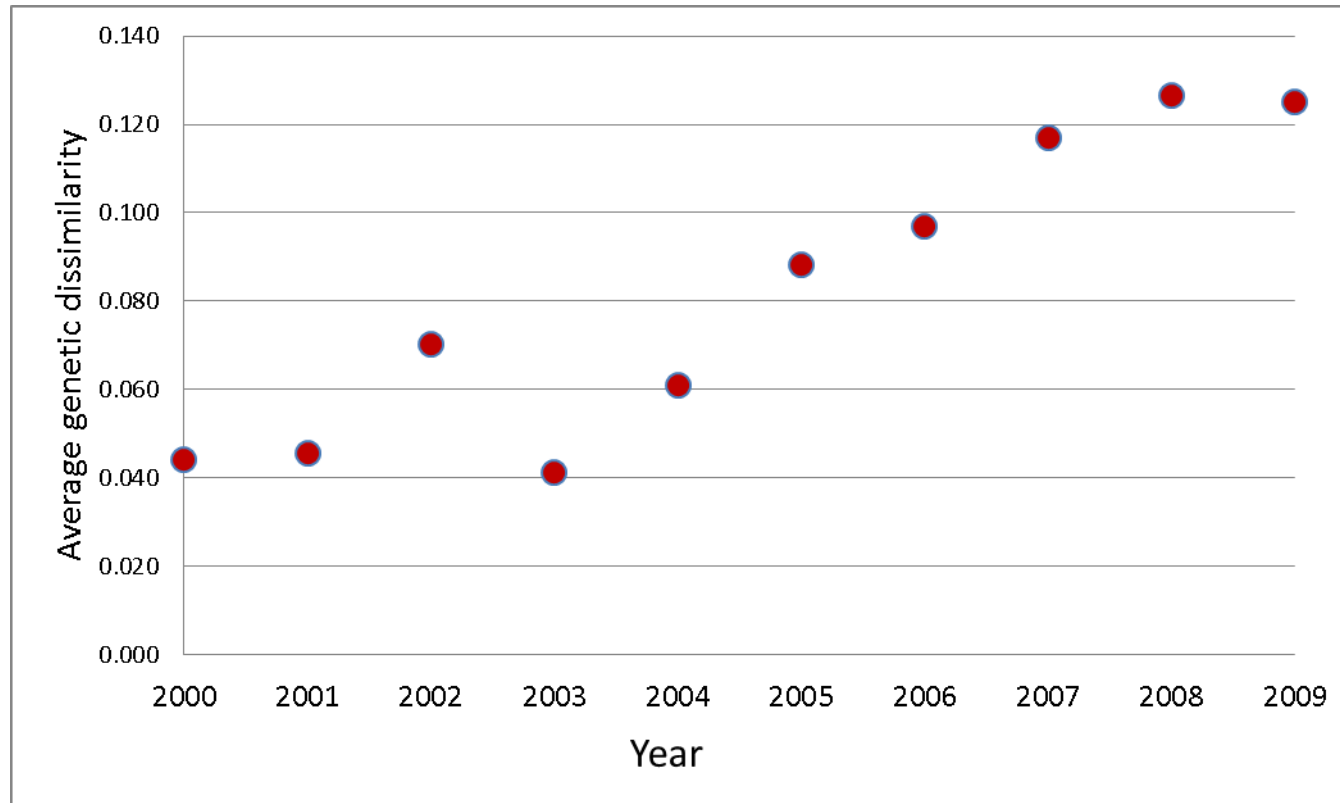
Over time course of epidemic radiating out in recent years, increasing diversity

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Bayesian skyline plot (Tim Vaughan)

Massive increase in bacterial population just prior to major human epidemic
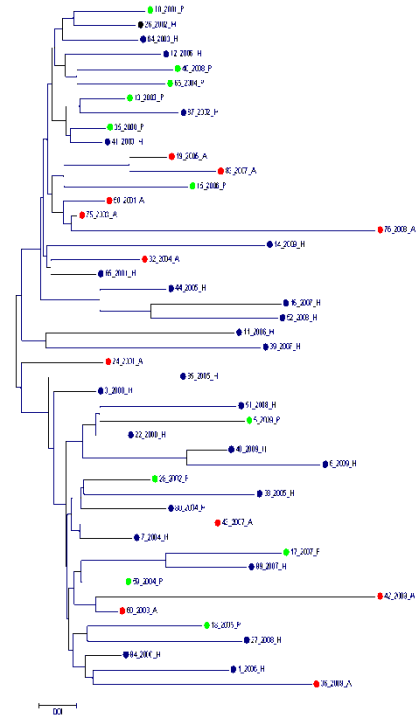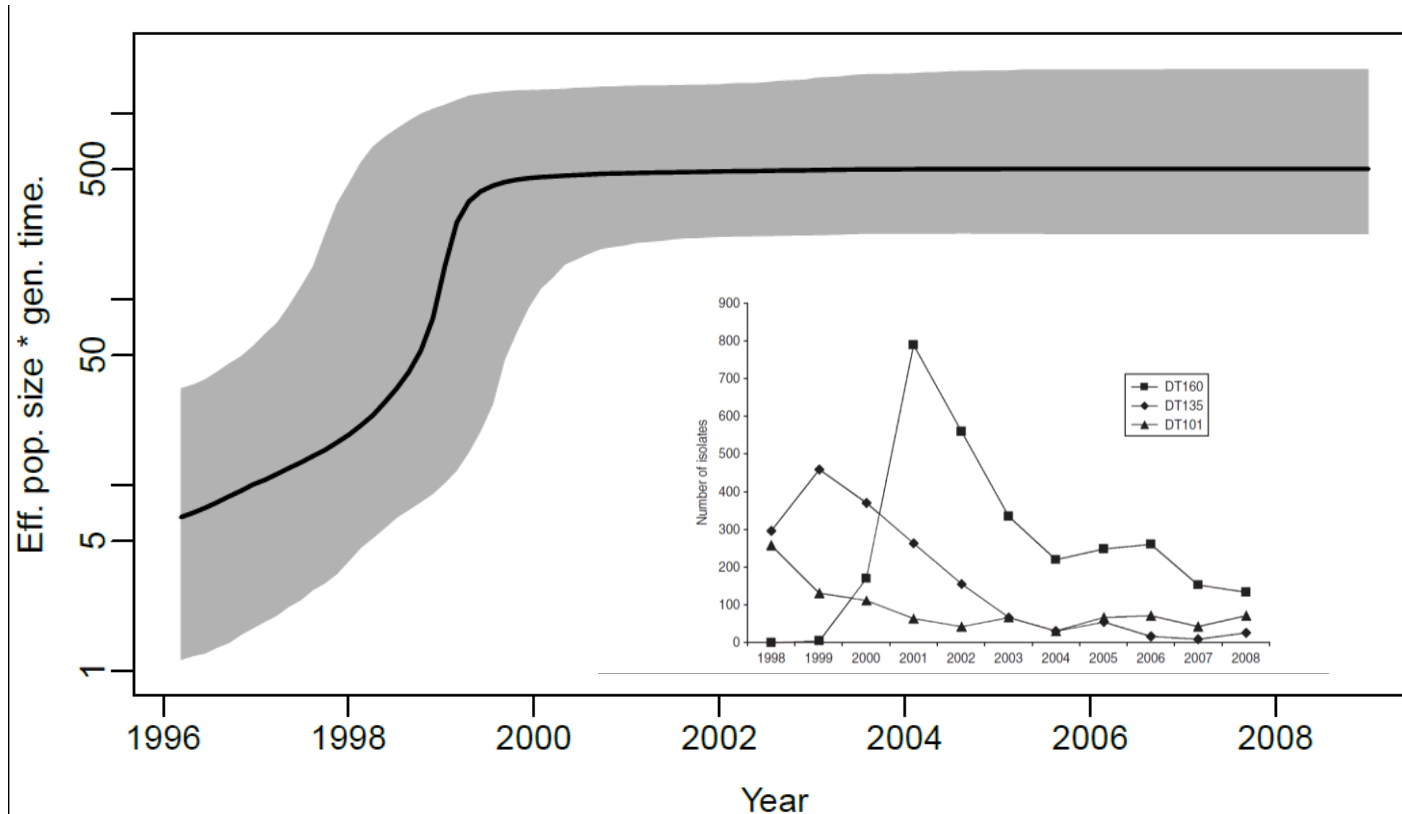
Increase in population size just prior to peak in human cases

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56 variant (RDNC May 06) evolved from DT160?
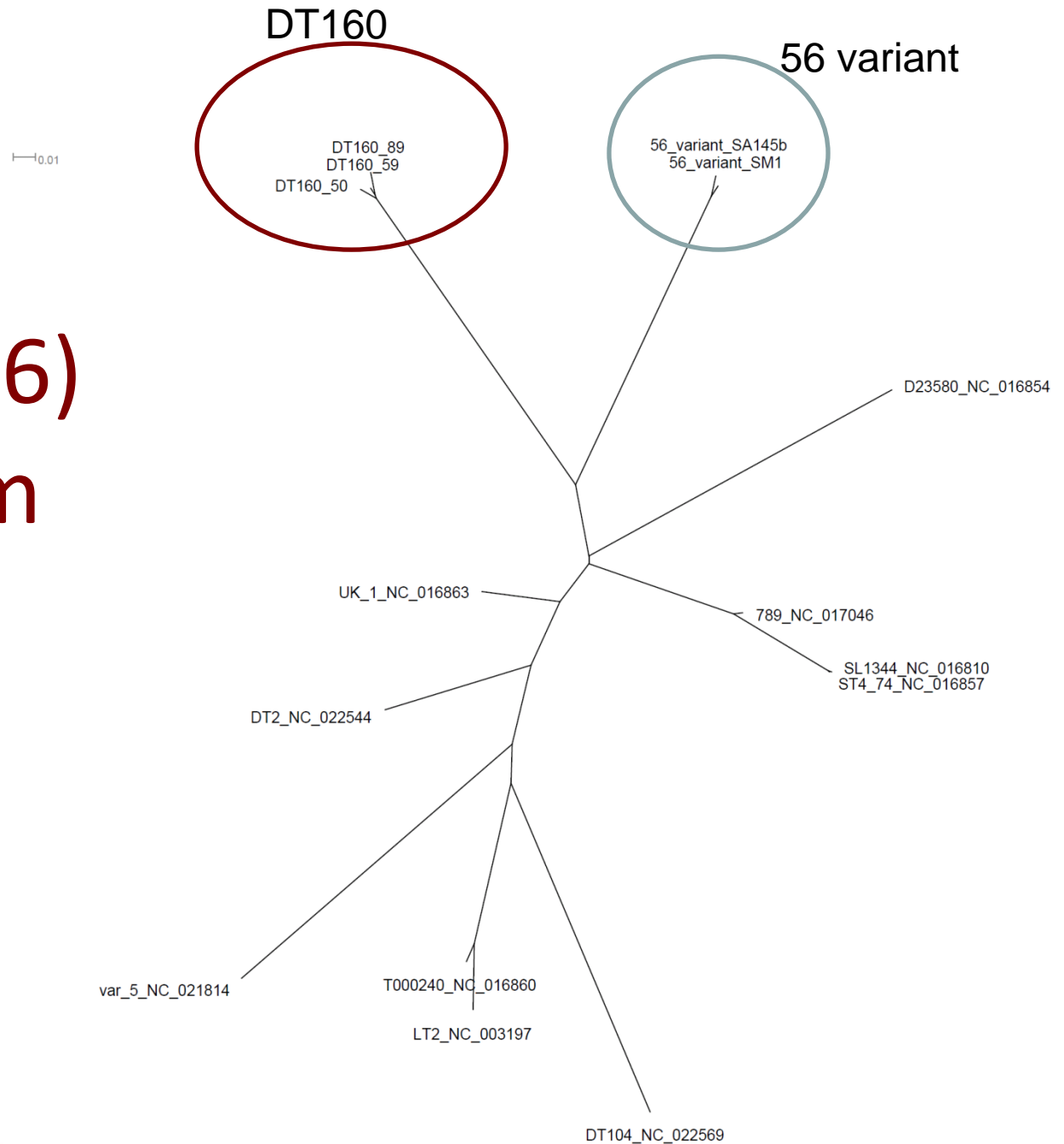
Host transitions- zoonotic or anthroponotic spread?

www.rndsystems.com

Distinguishable Epidemics of Multidrug-Resistant *Salmonella* **Typhimurium DT104 in Different Hosts**
A. E. Mather *et al.*
Science **341**, 1514 (2013);
DOI: 10.1126/science.1240578

Fore
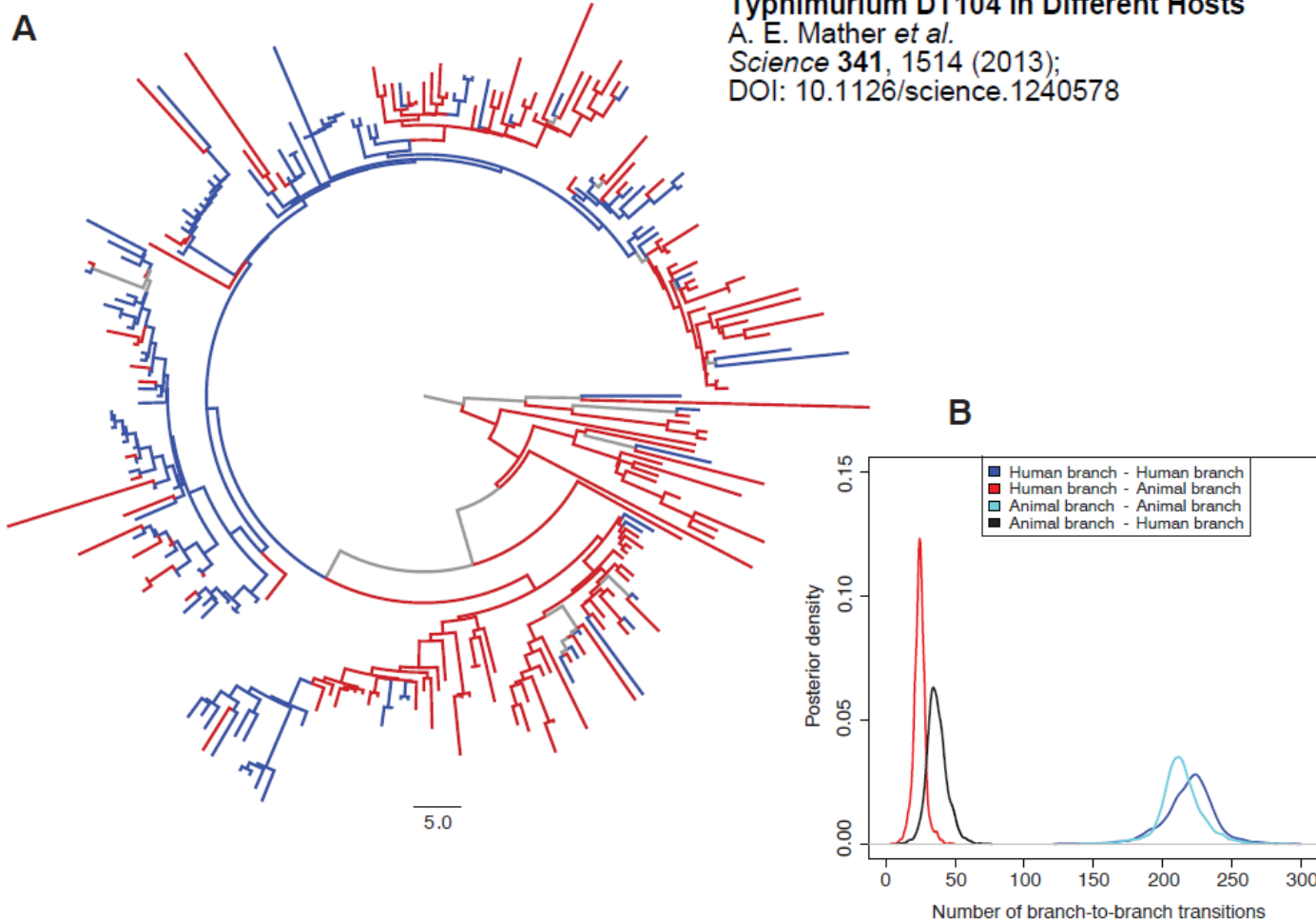
Conclusions: how will it help inform policy?

- Transmission
  - Speed of spread Ro, what vaccine coverage needed?
  - Transmission in (social) networks – who's infecting whom? (people and animals, conservation studies)
  - Importance of different transmission routes (zoonotic versus person to person?)

- Host switching
  - When, where, which way?

- Phenotype:genotype relationships
  - Refining source/reservoir attribution?
  - Diagnostics

- Number and origin of introductions
  - Informing biosecurity (where come from, how often)

- Evolution
  - Impact of interventions such as vaccination
  - Effects of antimicrobial use
  - Prediction of emergent strains

Ongoing genomic epi studies in ^{m}EpiLab

STEC O157
Non-O157 STEC
Salmonella
Campylobacter
Leptospira
Enterococci

Patricia Jaros

e Kunenga
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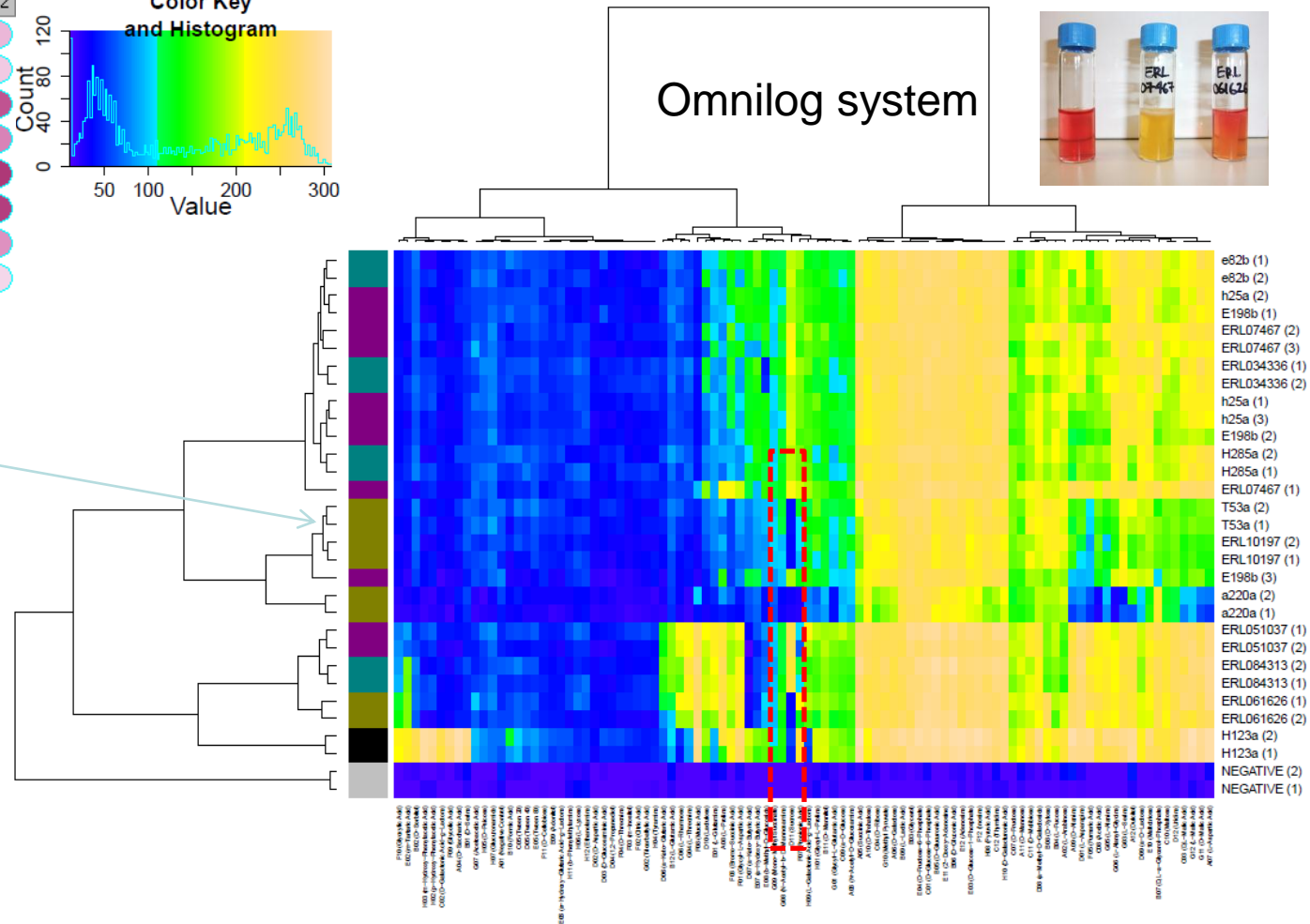# Campylobacter and STEC: genotype:phenotype studies

Tessy George

Omnilog system
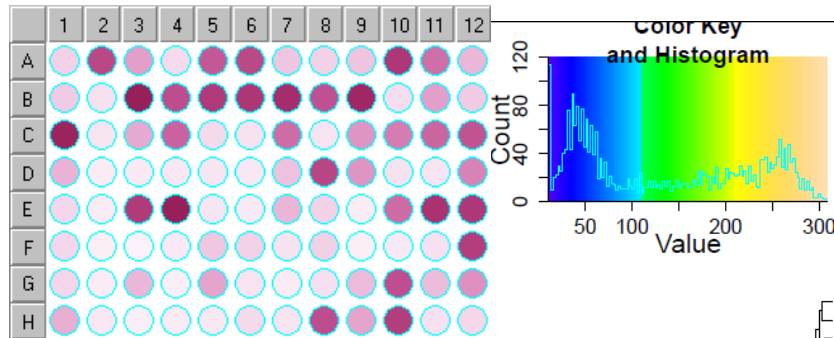
Brown SBI type 5 with stx_{2c}
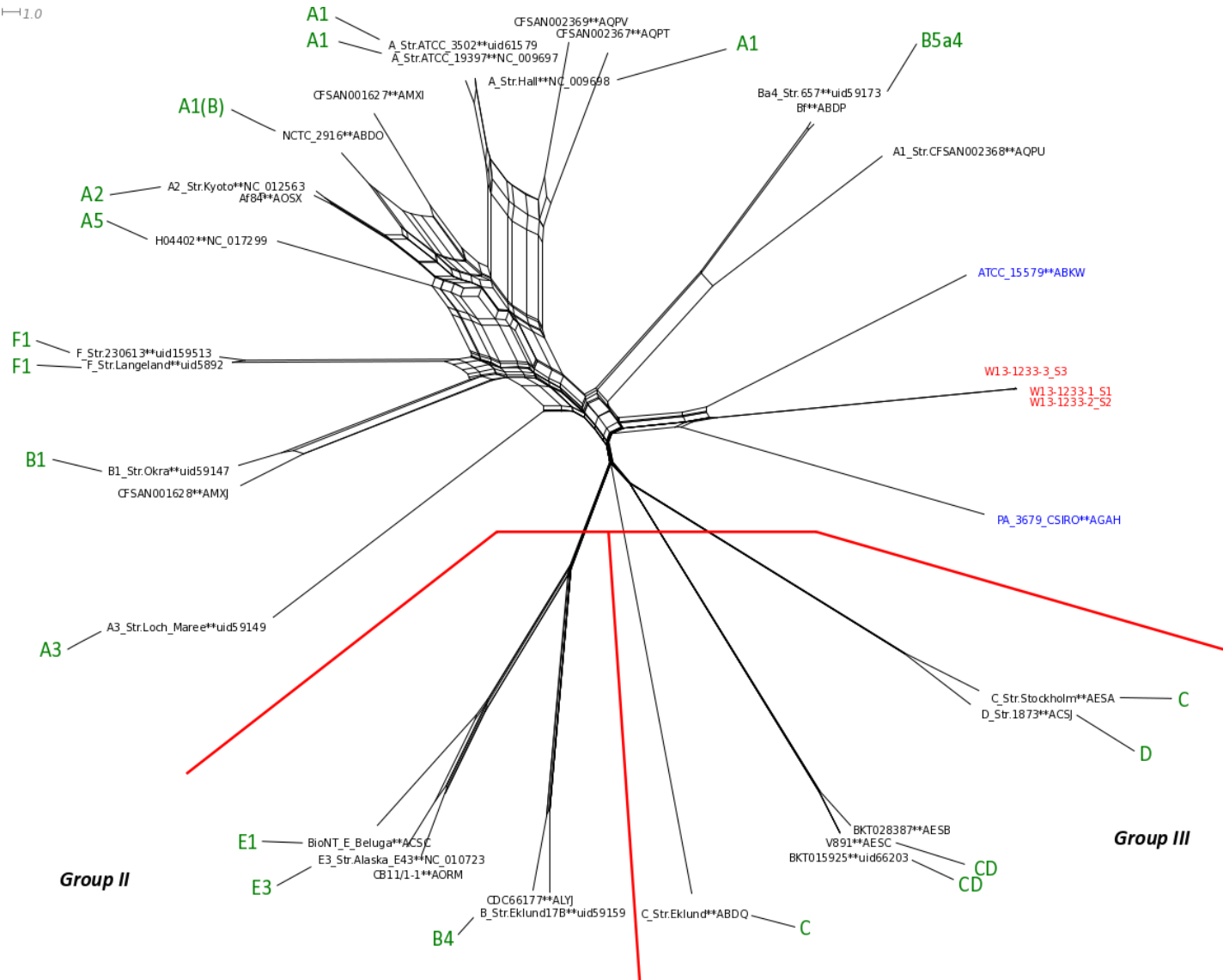
Sucrose non-fermenter

Other studies (Lee et. al AEM 2012) have shown $stx2c$ strains to be 'stress sensitive'

Food safety and the NZ economy*?*
Clostridium ….?

rMLST

Acknowledgements

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