The core genome and beyond: comparative bacterial pathogenomics and functional gene analysis in foodborne pathogens

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OIE Collaborating Centre for Veterinary Epidemiology and Public Health

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Acknowledgements

- members of mEpiLab, IVABS, Massey University
  - Lynn Rogers
- Massey Genome Service (MGS; a part of NZGL)
  - Lorraine Berry & Mauro Truglio
- Dept. of Zoology, University of Oxford, Oxford, UK
  - Keith Jolley & Martin Maiden
- Funding
  - Wellington Hospital Laboratory Education and Research Fund
  - IVABS, Massey University

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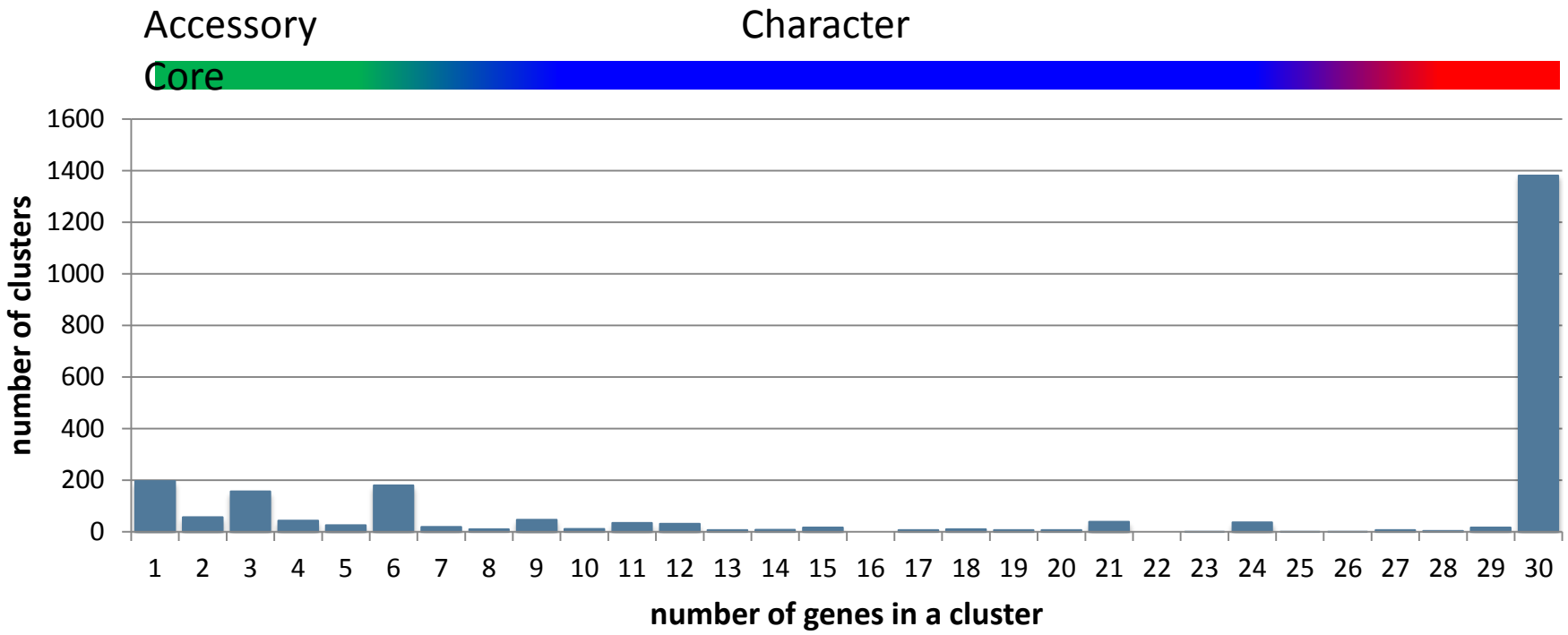
Overview

- The core genome
- Invasive isolates of *Campylobacter spp*.
- Comparative analysis
  - rMLST, core SNPs
  - Core genome and beyond
- COG classification for functional analysis
  - “biological weighting”

The core genome

- Genes present in ALL strains are core genes
  - A proxy for analysing evolution
- Genes present in a subset of strains are accessory genes
  - Virulence?
  - Niche adaptation?



Clinical observations

- 10 invasive isolates from Wellington Hospital from 2010 to 2012:
  - Aged 19 to 89 years
  - 6 presented with diarrhoea
  - Others
    - Headache
    - Prosthetic hip infection
    - Exacerbation of chronic pulmonary disease
  - 9 samples from blood
  - 1 sample from joint aspirate
- Outcomes:
  - 6/10 treated with oral ciprofloxacin
  - 43 year old died
  - One had episode 4 years earlier

Campylobacter and bacteraemia

- Campy bacteraemia appears to originate from acute colitis
- Bacteraemia relatively subtle part of illness
  - Only one person with sepsis syndrome
- Complicated infection with discitis or prosthetic joint infection suggestive of acute seeding of previously abnormal tissue

- Bacteraemia population rate = 7.6 per million person years
  - Denmark = 2.9 per million person years[1]
- Bacteraemia:enteritis ratio = 0.4%
  - Finland = 0.3%[2]

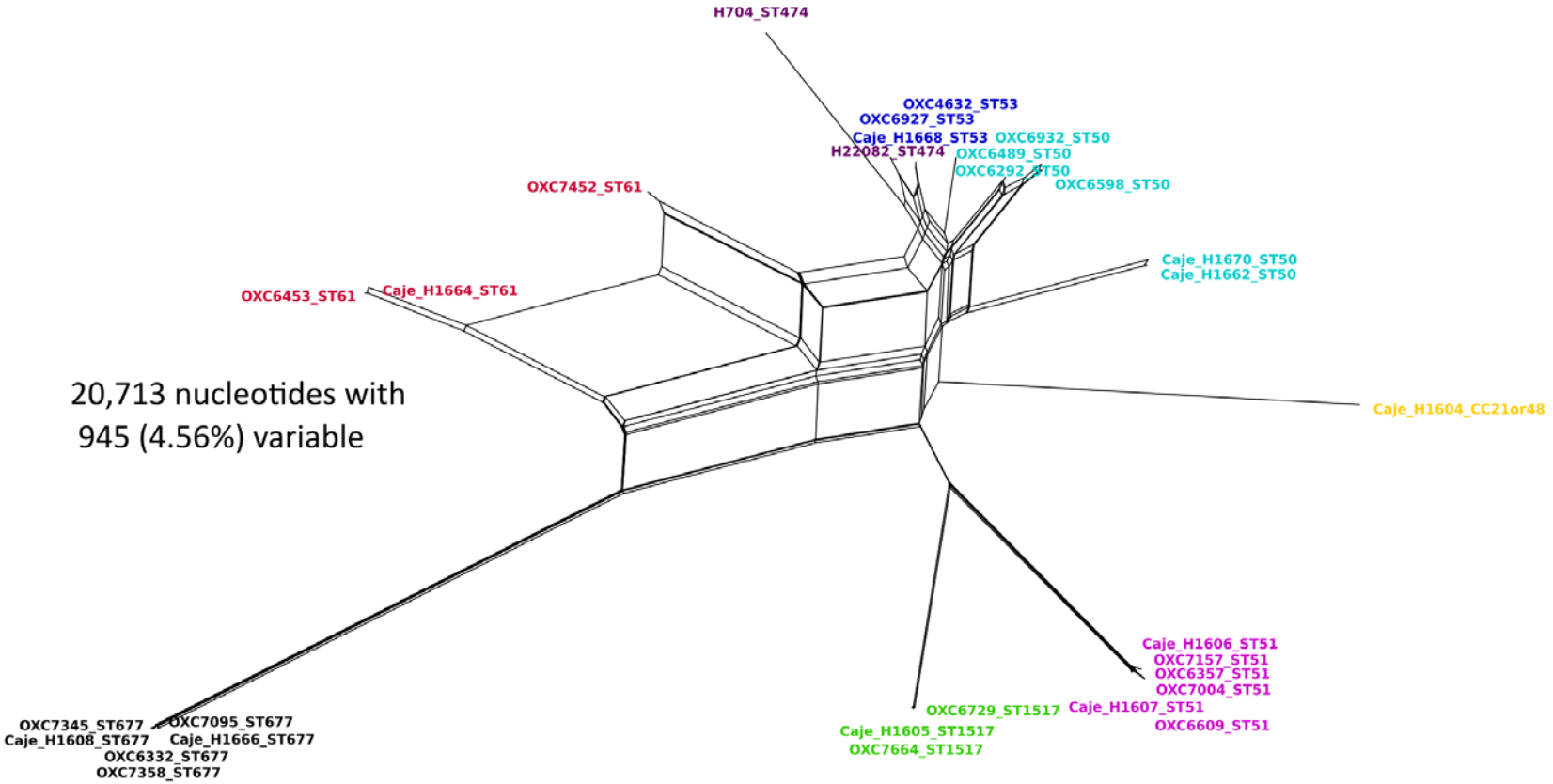
1: Nielsen et al. Clin Microbiol Infect 2010; 16: 57–61
2: Feodoroff et al. Clinical Infectious Diseases 2011;53(8):e99–e106

Genome summaries

- MLST results:
  - 5 clonal complexes found:
    - 2 from CC677, 2 from CC443, 4 from CC21, 1 from CC354 and 1 from CC61
- For comparison, 2 random isolates taken per ST from the Oxford surveillance project (OXC)
- Genomes:
  - When compared to the 20 random chosen OXC genomes:
  - No overall difference in:
    - Overall genome size: 1.654 Mb (± 22kb) vs. 1.659 Mb (± 14kb)
    - GC content: 30.38% (± 0.06%) vs. 30.38% (± 0.05%)
    - Number of predicted genes: 1705 (± 30) vs. 1714 (± 18)
  - Difference in:
    - tRNA number: 42.1 (± 1.16) vs. 37.3 (± 2.21)
    - but these are probably due to *de novo* assembly issues

Results – ribosomal MLST (rMLST[3])

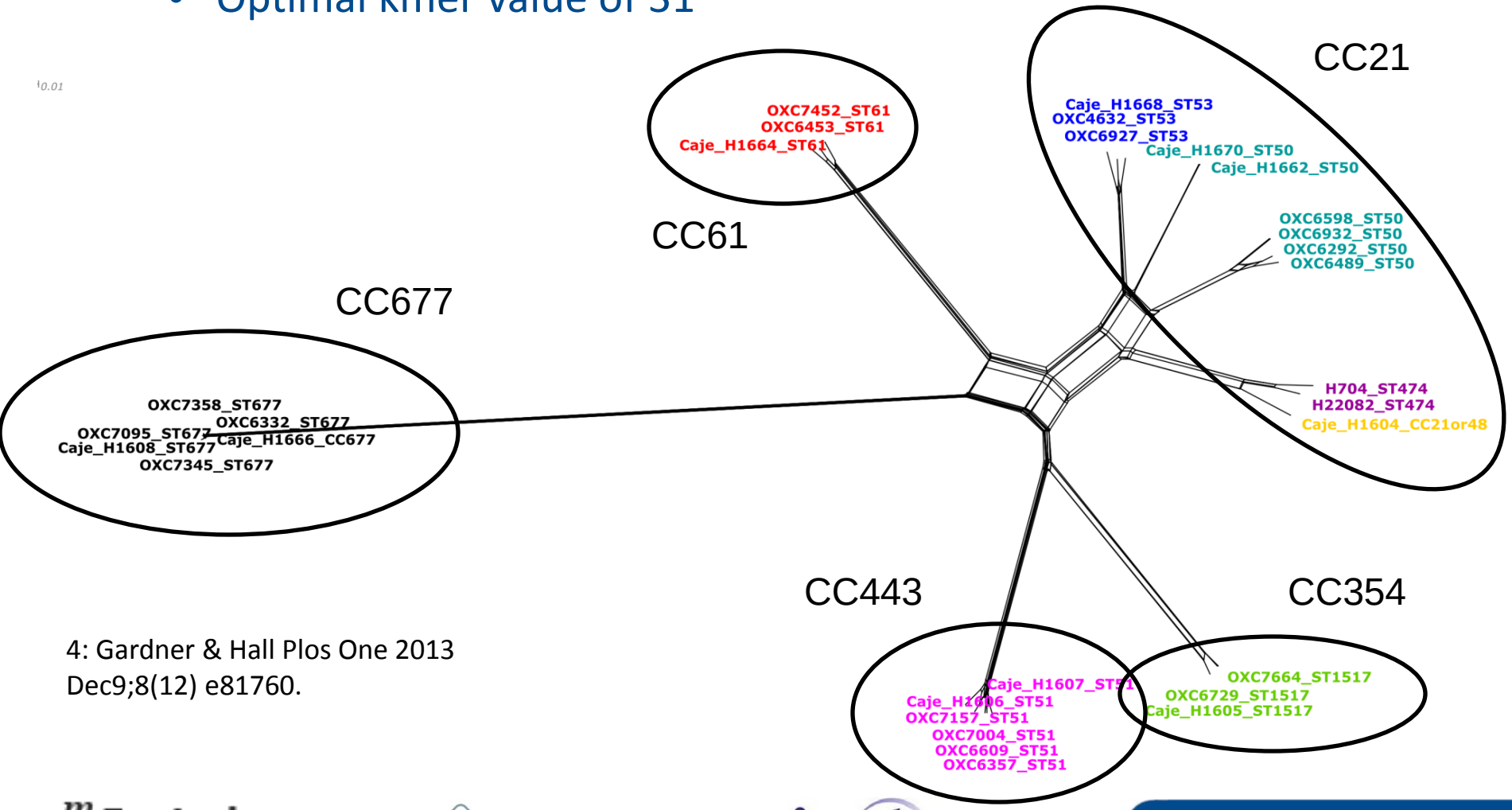
- 52 genes (no *rpm*D in order Campylobacterales)



3: Jolley et al. Microbiology 2012 Apr;158(Pt 4):1005-15

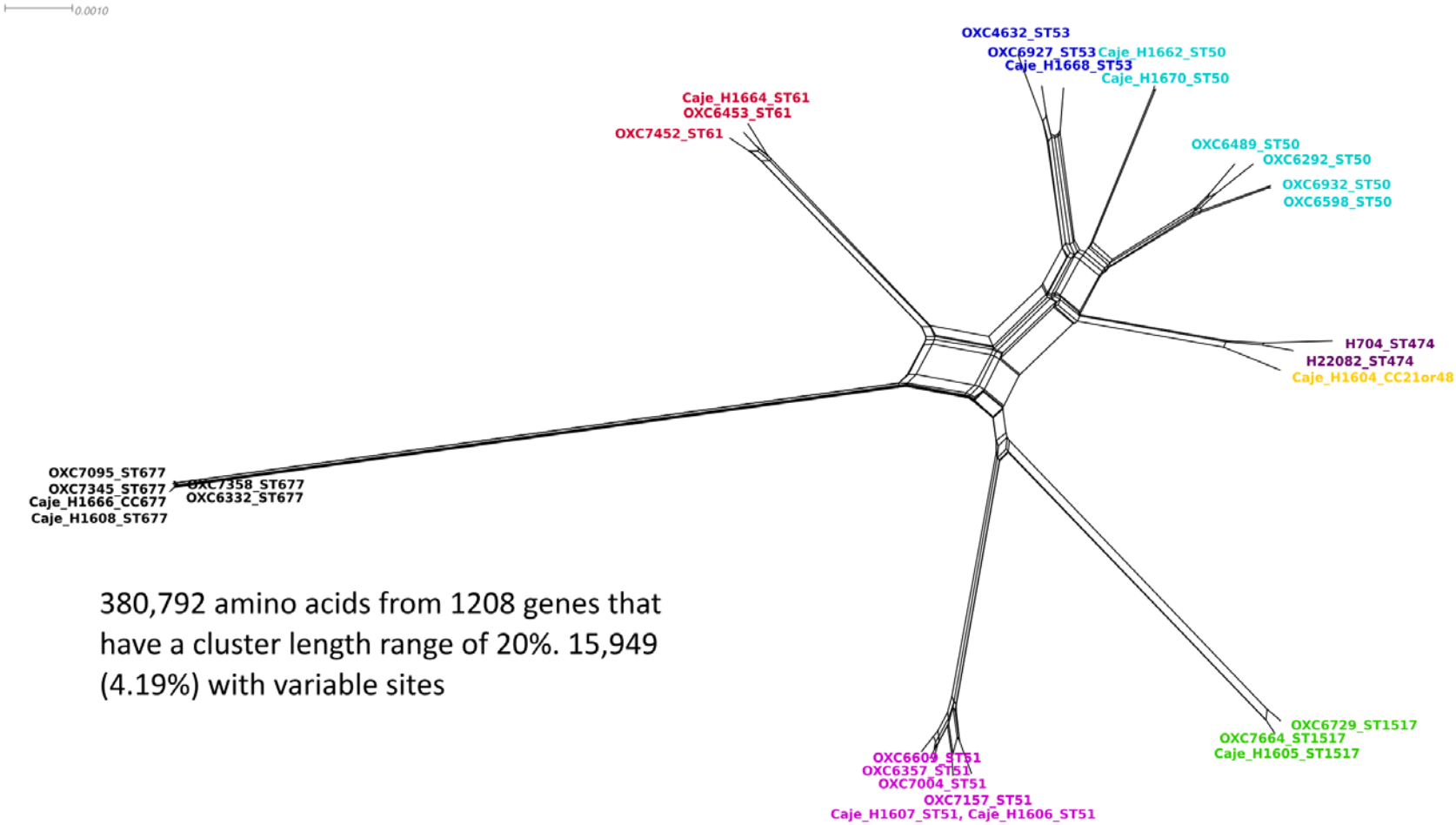
Results – core SNPs

- e.g. kSNP2[4] analysis based on 13,260 core SNPs
  - Optimal kmer value of 31



4: Gardner & Hall Plos One 2013 Dec9;8(12) e81760.

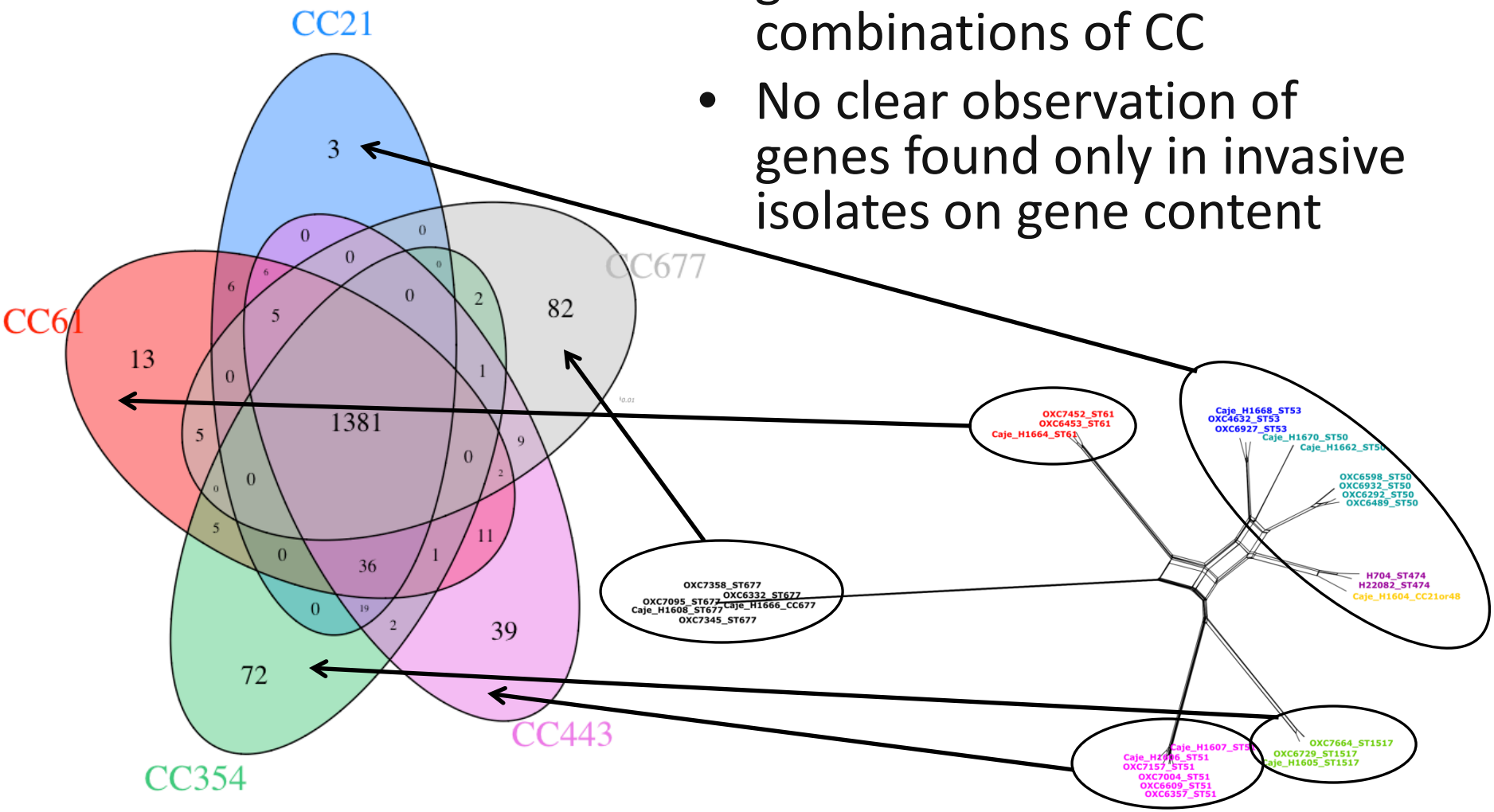
Results – core genome



380,792 amino acids from 1208 genes that have a cluster length range of 20%. 15,949 (4.19%) with variable sites

Beyond the core

- From OrthoMCL, different genes are found in different combinations of CC
- No clear observation of genes found only in invasive isolates on gene content



Functional analysis by COGs

- Clusters of Orthologous Groups – COGs
- 1997 - concept by Koonin et al. as a framework for functional and evolutionary genome analysis[5]
- Hierarchical process wherein:
  - Gene function defined into 26 codes (A to Z)
    - e.g. P = 'Inorganic ion transport and metabolism'
  - Each of the 4632 curated orthologous group of genes gets a COG classification, and with it a functional code
    - e.g. COG2847 = 'Copper(I)-binding protein'
- Idea: use COGnitor software to analyse the function of predicted genes

5: Tatusov et al. Science 1997 Oct 24;278(%338):631-7

Whole genome analysis by function

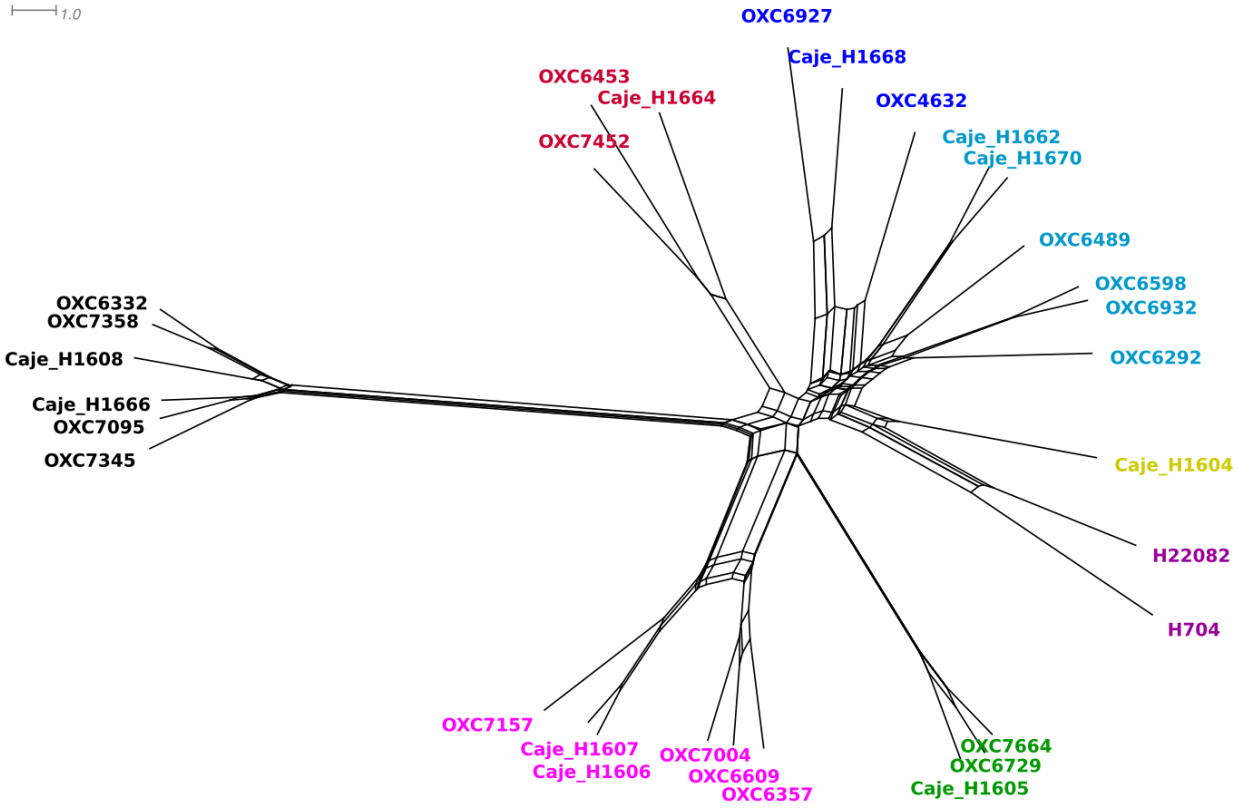
- Allows analysis of complete dataset – i.e. not just the core genome
- Analysis as a two step process:
  - For each isolate under investigation:
    - BLAST the PROKKA gene predictions against COG database of ~1.8 million genes using PSIBLAST
    - Parse output with COGnitor and generate counts for each COG in the genome
  - For all isolates:
    - Detect COGs where there is a difference in number across isolates
    - Use these values to generate a distance matrix
    - Convert to Nexus format and view resulting tree as a NeighborNet in SplitsTree

Insights from function?

- Is the difference between absence/presence more important than that between the number of paralogues?
  - i.e.: should 1,0,0,1,2,5 be recoded as:
    - binary (1,0,0,1,1,1),
    - something else e.g. 1,0,0,1,1.3,1.7?
- Can look at this by changing weighting of the data in the distance matrix
- Values of weight *w* can vary between 0 and 1:
  - $w = 0$
    - Data is binary, i.e. absence or presence of a COG member
  - $w = 1$
    - Data has its values, i.e. 4 paralogues of a COG member provide 4x more weight than 1 member
- What is the value of *w* that is biologically relevant:
  - 0, 1, or somewhere in-between?

COG function – Euclidean (p = 2)

- What is the effect on genome datasets?
- Values of *w* varied from 0 to 1
- Correlation with ST rather than disease state



COG summary:
Absent: 3282
Present: 866
Variable: 484

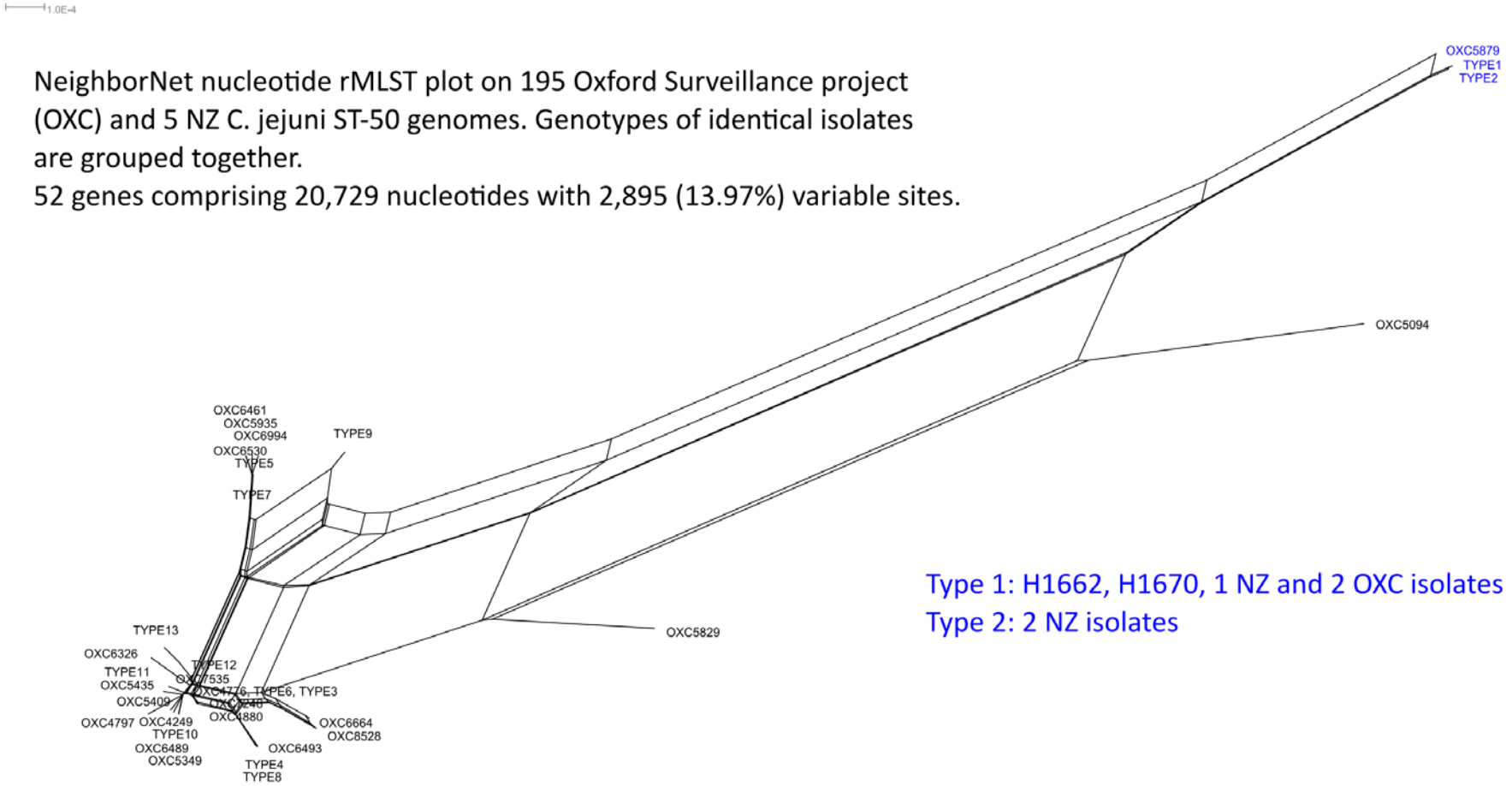
***w* = 1.00**

Summary

- 10 NZ invasive strains whole genome sequenced and 5 clonal complexes found
- Comparison with 20 OXC gastroenteritis isolates:
  - Isolates grouped by sequence type rather than phenotype at levels of:
    - rMLST, core SNP, core genome and COG function
- Differences in phenotype of invasive isolates are not at overall level of genome
  - Further work required to investigate the role of sequence differences

ST50 – slightly different

NeighborNet nucleotide rMLST plot on 195 Oxford Surveillance project (OXC) and 5 NZ C. jejuni ST-50 genomes. Genotypes of identical isolates are grouped together.
52 genes comprising 20,729 nucleotides with 2,895 (13.97%) variable sites.



Type 1: H1662, H1670, 1 NZ and 2 OXC isolates
Type 2: 2 NZ isolates

Summary

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- Comparison with 20 OXC gastroenteritis isolates:
  - Isolates grouped by sequence type rather than phenotype at levels of:
    - rMLST, core SNP, core genome and COG function
- ST50 isolates from NZ show slight difference to initial OXC isolates
  - Further rMLST analysis showed most OXC ST50 isolates (193/200) are unlike NZ isolates
- Differences in phenotype of invasive isolates are not at overall level of genome
  - Further work required to investigate the role of sequence differences