Understanding the emergence of Group B Streptococcus neonatal infections by the genomic analysis of today's and collection isolates

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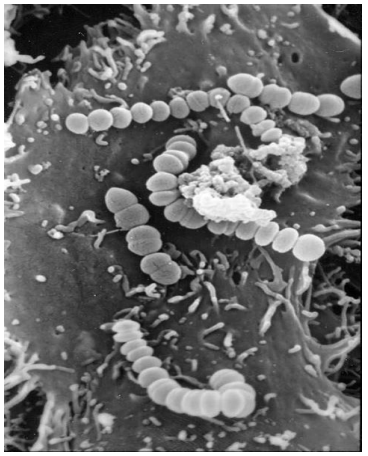
S. agalactiae or group B streptococcus (GBS)

- Opportunistic pathogen
- Human
  - Commensal of the digestive or urinary tract
  - Leading cause of neonatal infections
  - Emerged during the 1960s–1970s
  - Risk for immuno-compromised adults and elderly



- Broad host spectrum in animals
  - Udder infections in bovines and camels
  - Invasive diseases in fish - outbreaks

The emergence of GBS neonatal infections

Dtsch med Wochenschr 1965; 90(6): 258-261
DOI: 10.1055/s-0028-1111328

Streptococcus agalactiae als Erreger von Säuglingsmeningitiden

Streptococcus agalactiae as a cause of meningitis in the newborn (report of three cases)

G. Kexel, S. Schönbohm

Summary

Within **half a year three** independent cases of **meningitis** caused by *Streptococcus agalactiae* were observed (at the Children's Clinic, University of Bonn). The three infants were **under the age of three months** and the disease took a fulminating course leading to **death within hours**. The clinical picture of the three cases was very similar and was different from that observed in other types of meningitis. Bacteriological differention of the causative organism is discussed. **The manner of infection remains unexplained.**

MLST versus Genome sequencing

MLST

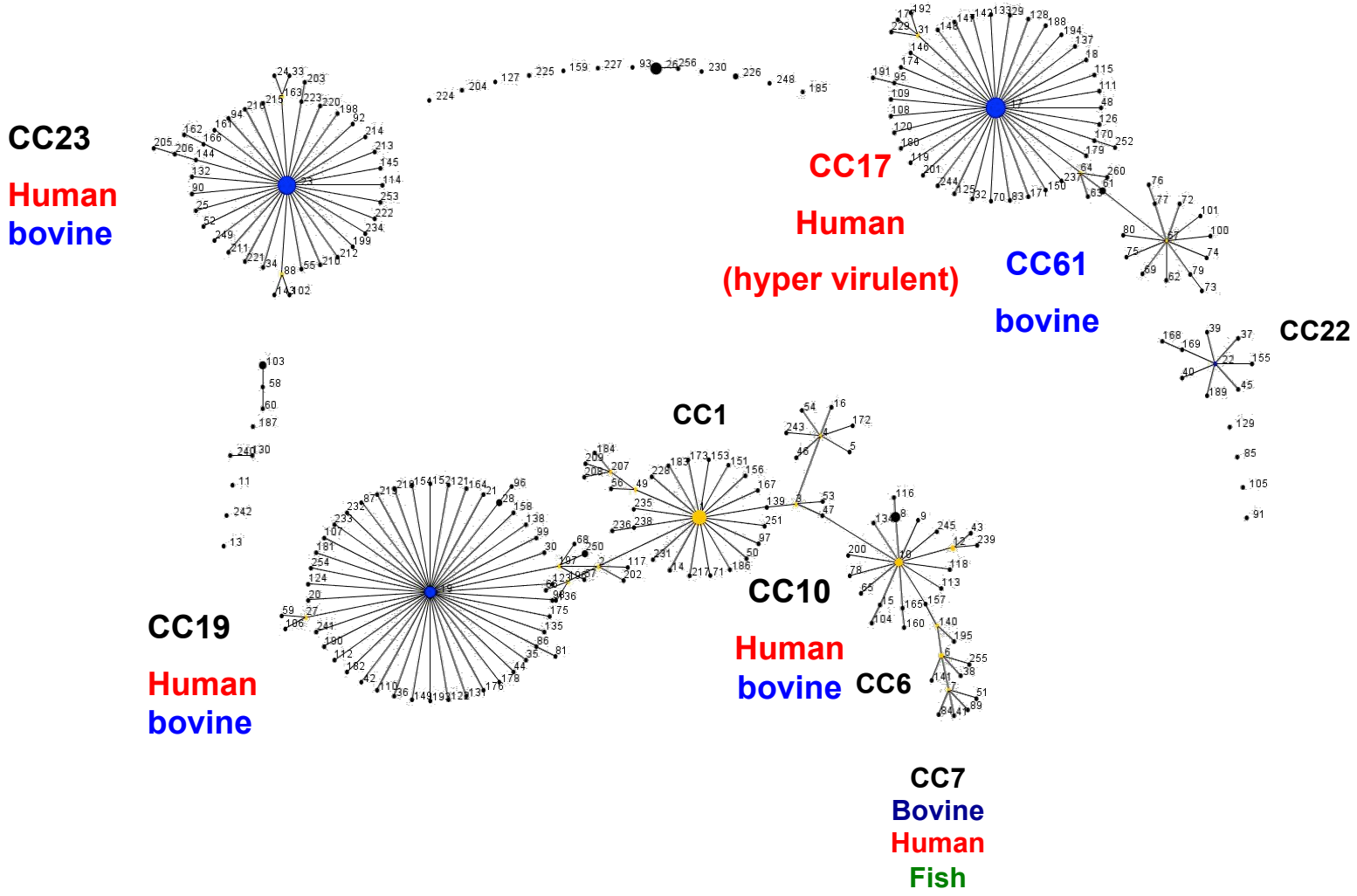
- Based on the sequences of 7 genes
- 751 Sequence Types (ST)
- Performed by 57 laboratories from 29 countries
- No redundancy
- Easy to analyse but low resolution

Genome sequencing

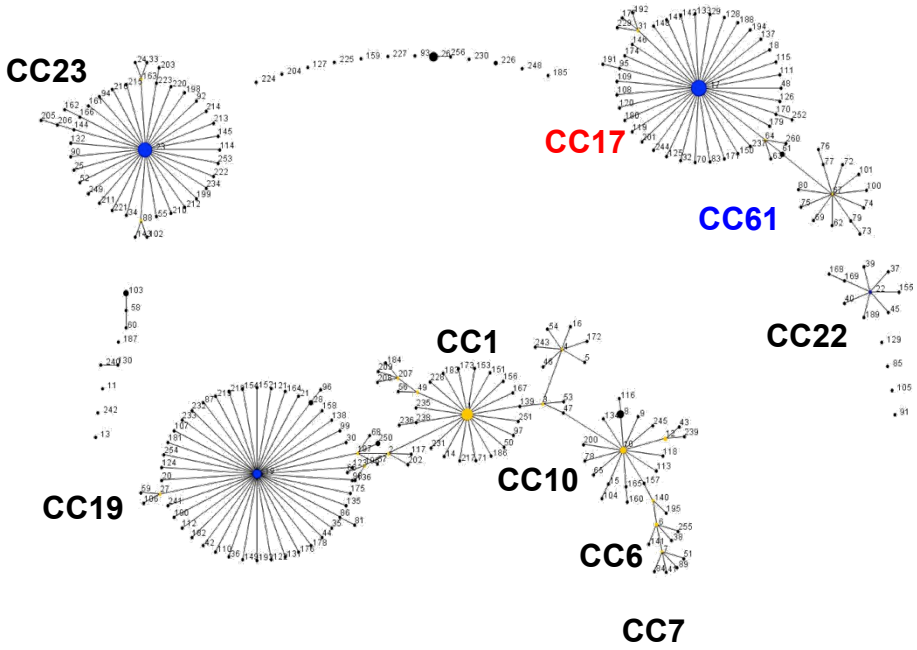
- Complete genomic information
- 506 available genome sequences
- Performed by few laboratories
- Specific sampling to respond specific questions

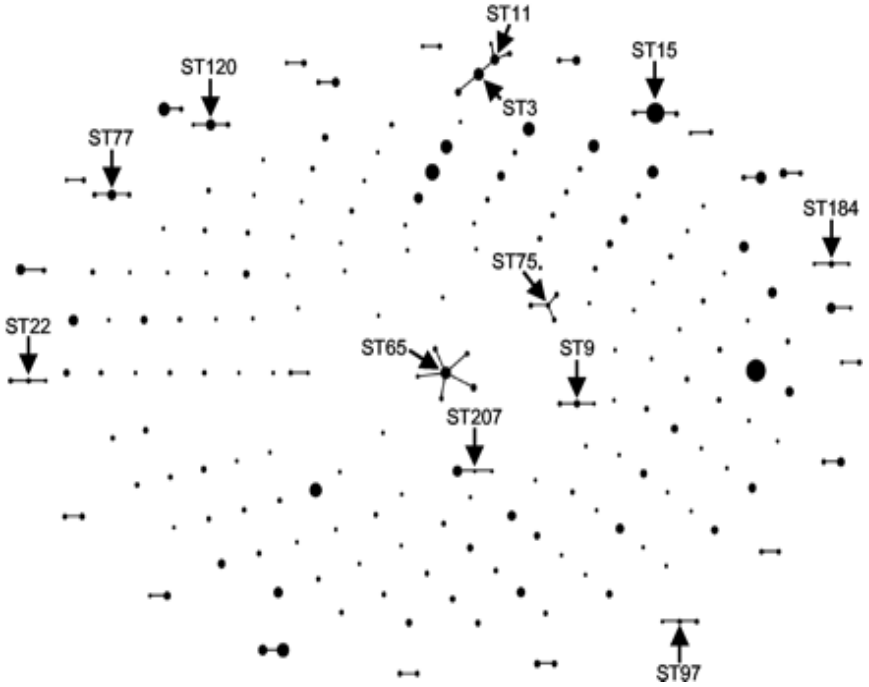
Population structure of GBS

derived from MLST



GBS versus GAS population structures



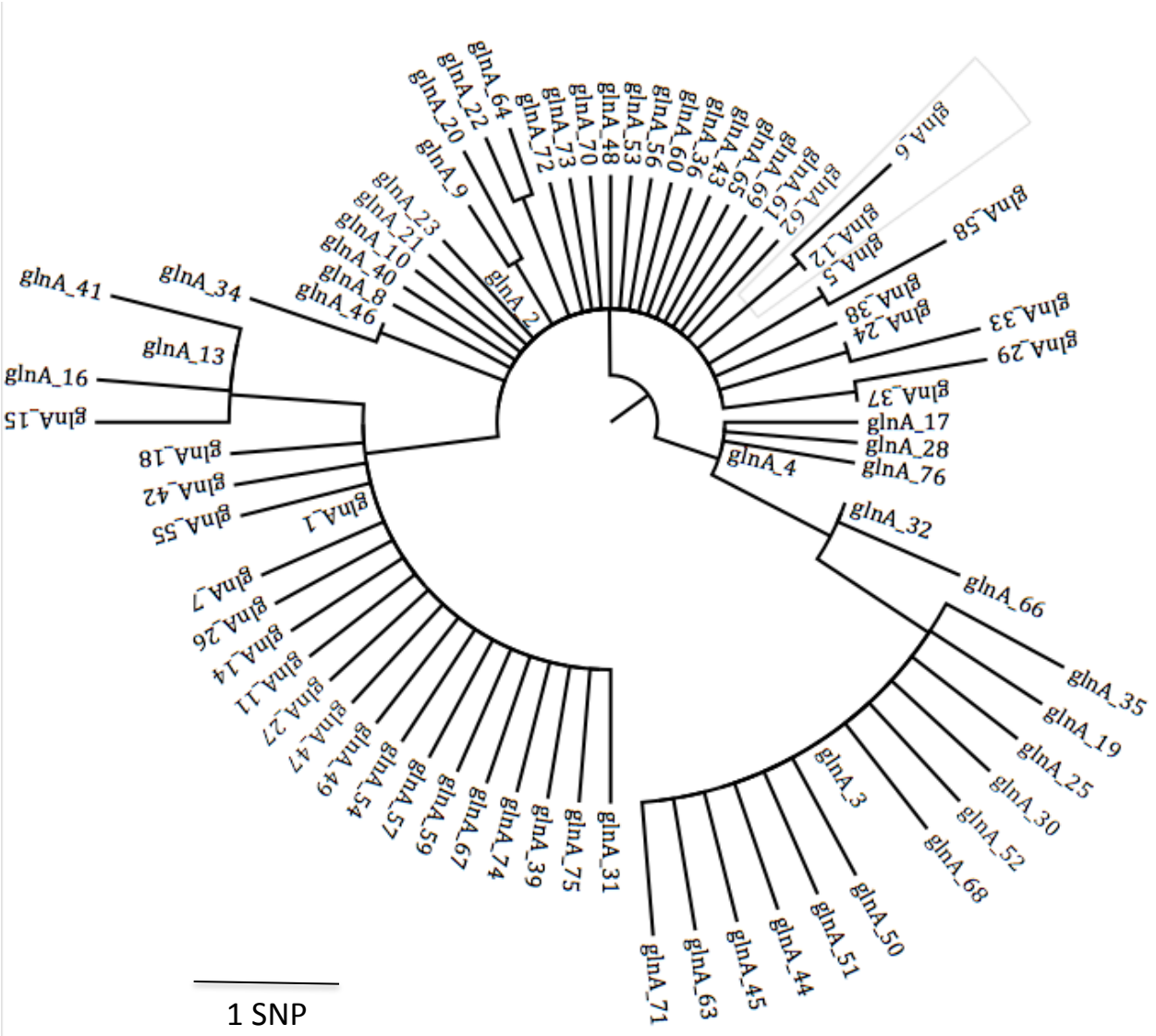


⇒ Low complexity of GBS population

Population studies of GBS

Study	Origin	Nb of isolates	CC1 (ST1)	CC8-10	CC17	CC19 (ST19, 28)	CC23	CC26	other
Jones	World	152	16% 14%	18%	30%	17% 13 - 2%	12%	2%	4%
Luan	Sweden	158	15% 9%	13%	24%	29% 20%	14%	0	4%
Manning	USA	338	29% 25%	18%	8%	17% 14%	25%	0,3%	2%
Bohnsack	Canada	899	16% 10%	9%	10%	17% 12%	40%	0	4%
Sadowi	Poland	112	18% 13%	18%	14%	13% 9 - 2%	37%	0	0
Huber	Kenya	169	12% 9%	17%	21%	13% 5%	27%	2%	7
Brochet	Dakar Bangui	163	20% 9%	6%	12%	28% 4% - 15%	17%	15%	2

New STs and clonal complexes

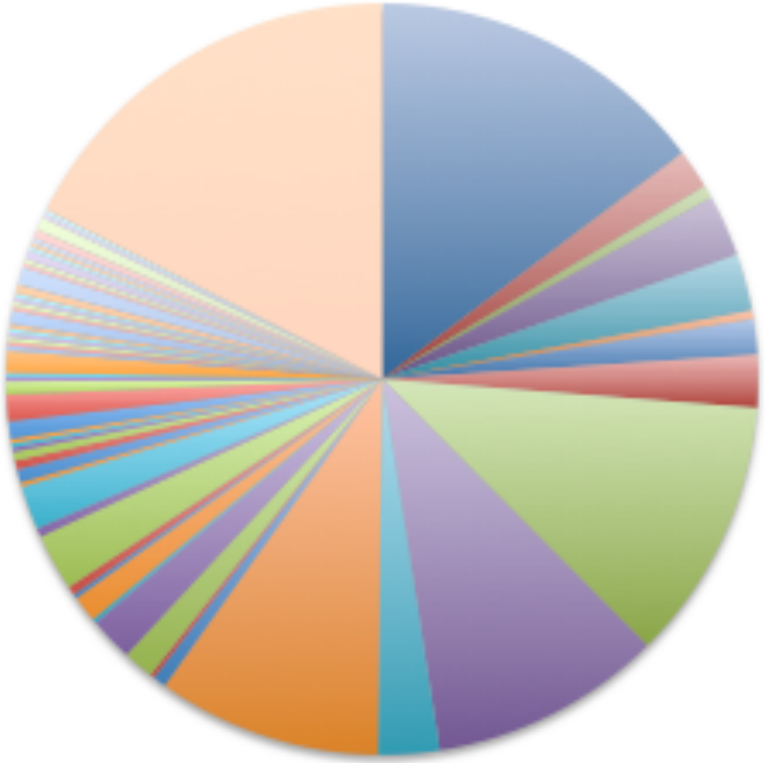


glnA ML tree
75 alleles

All recent alleles show single SNPs with older alleles

- Most of the phylogenetic diversity of the species is known
- New combination of STs

Distribution of genome sequences in STs

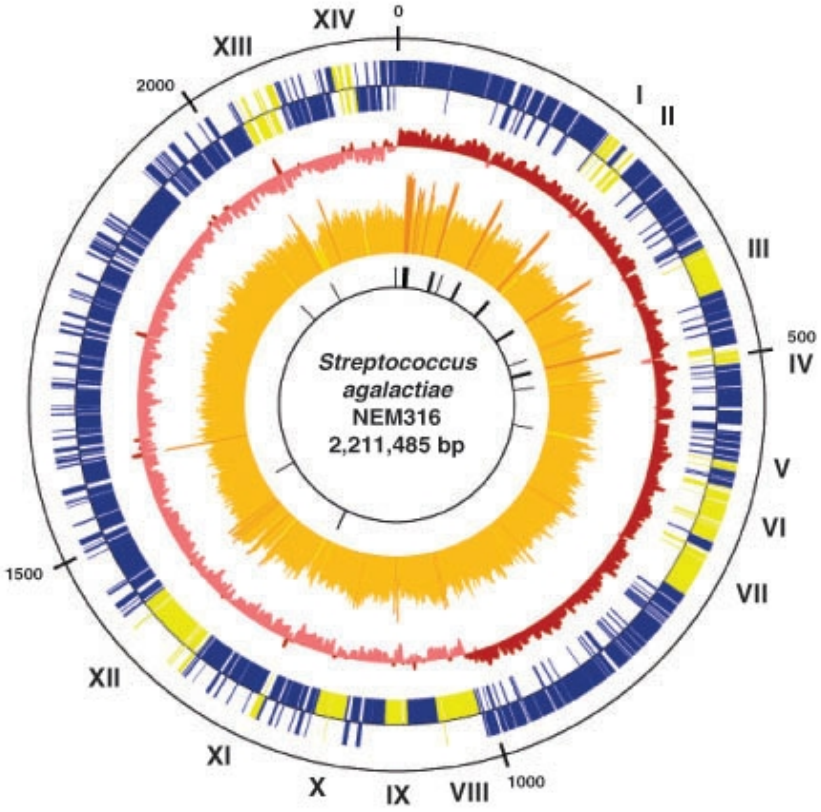


Belong to 59 STs

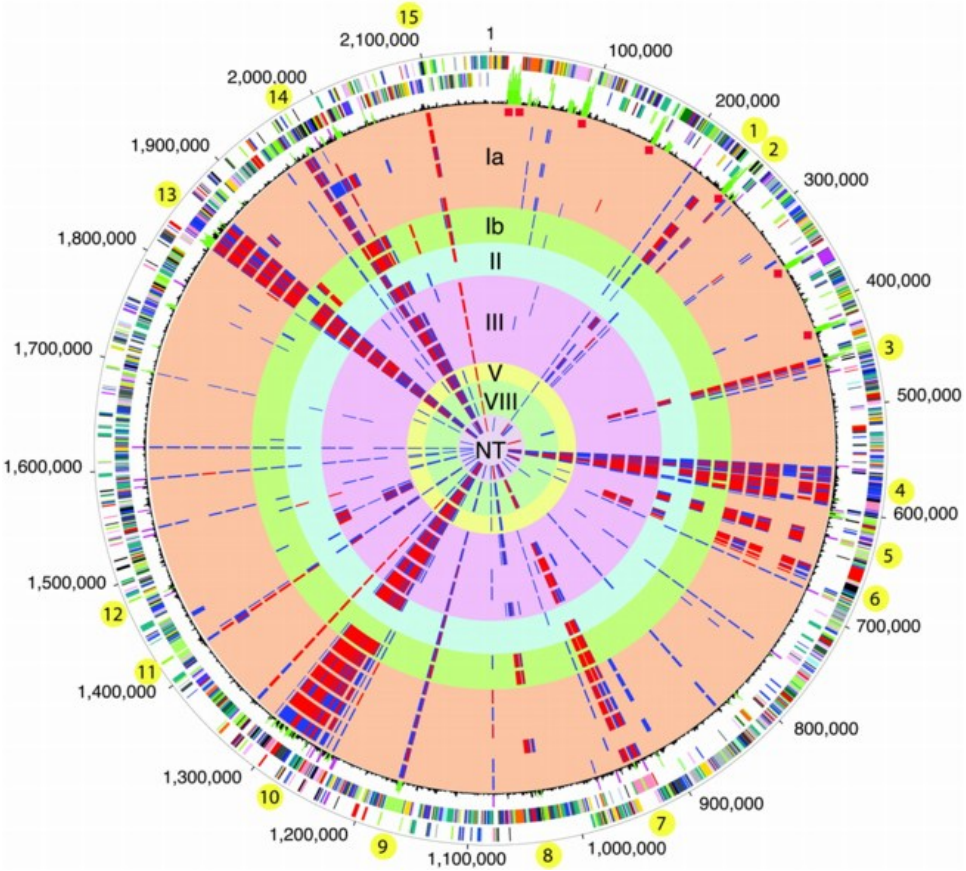
ST1:	78
ST7,8,10, 12:	47
ST17:	60
ST19:	53
ST23:	51
Unidentified:	93

- Complete genome sequencing covers only partially the diversity of the species
- Sequencing of specific isolates to fill gaps in the diversity

Sequencing 1 genome: a mosaic organization

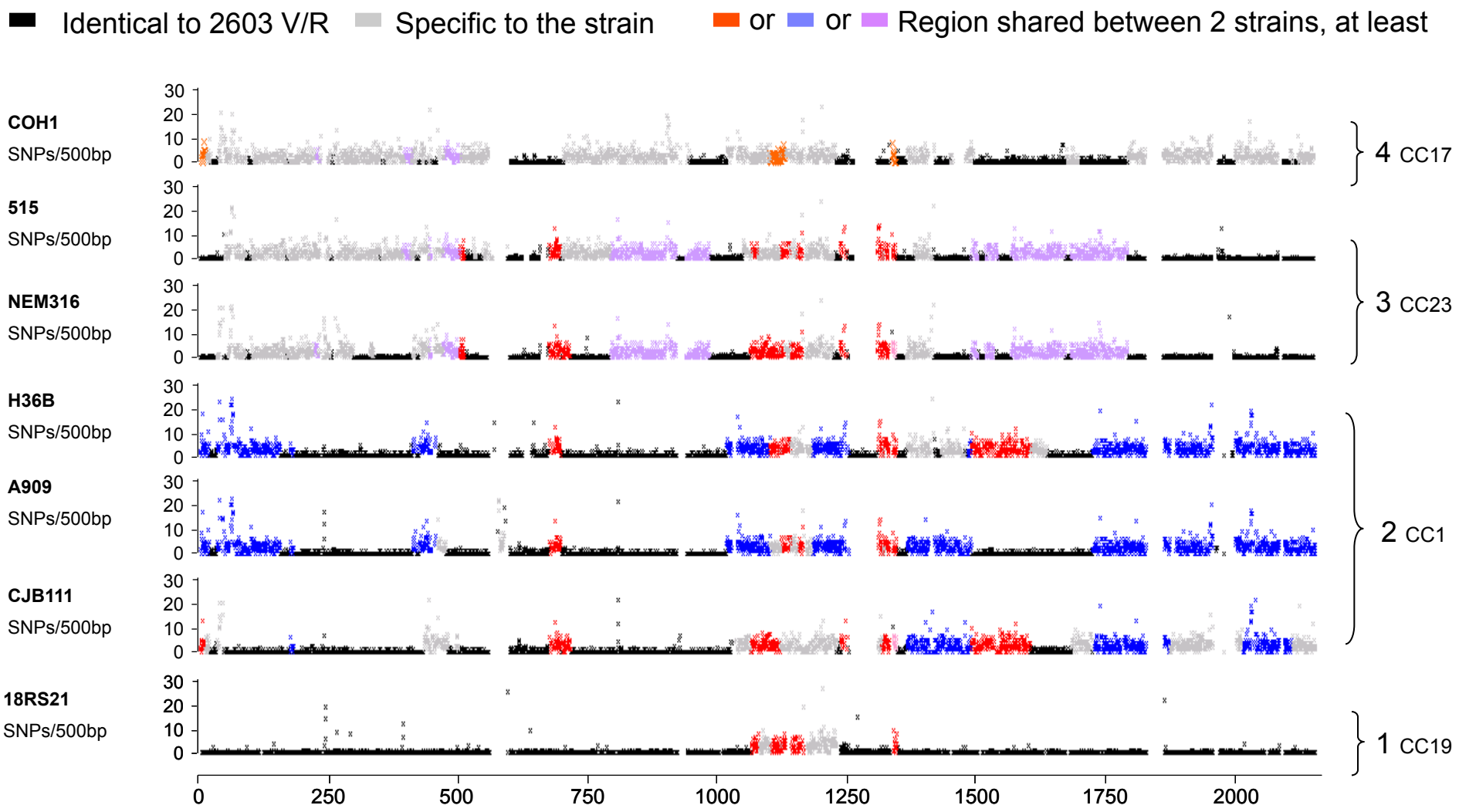


Glaser *et al.* 2002



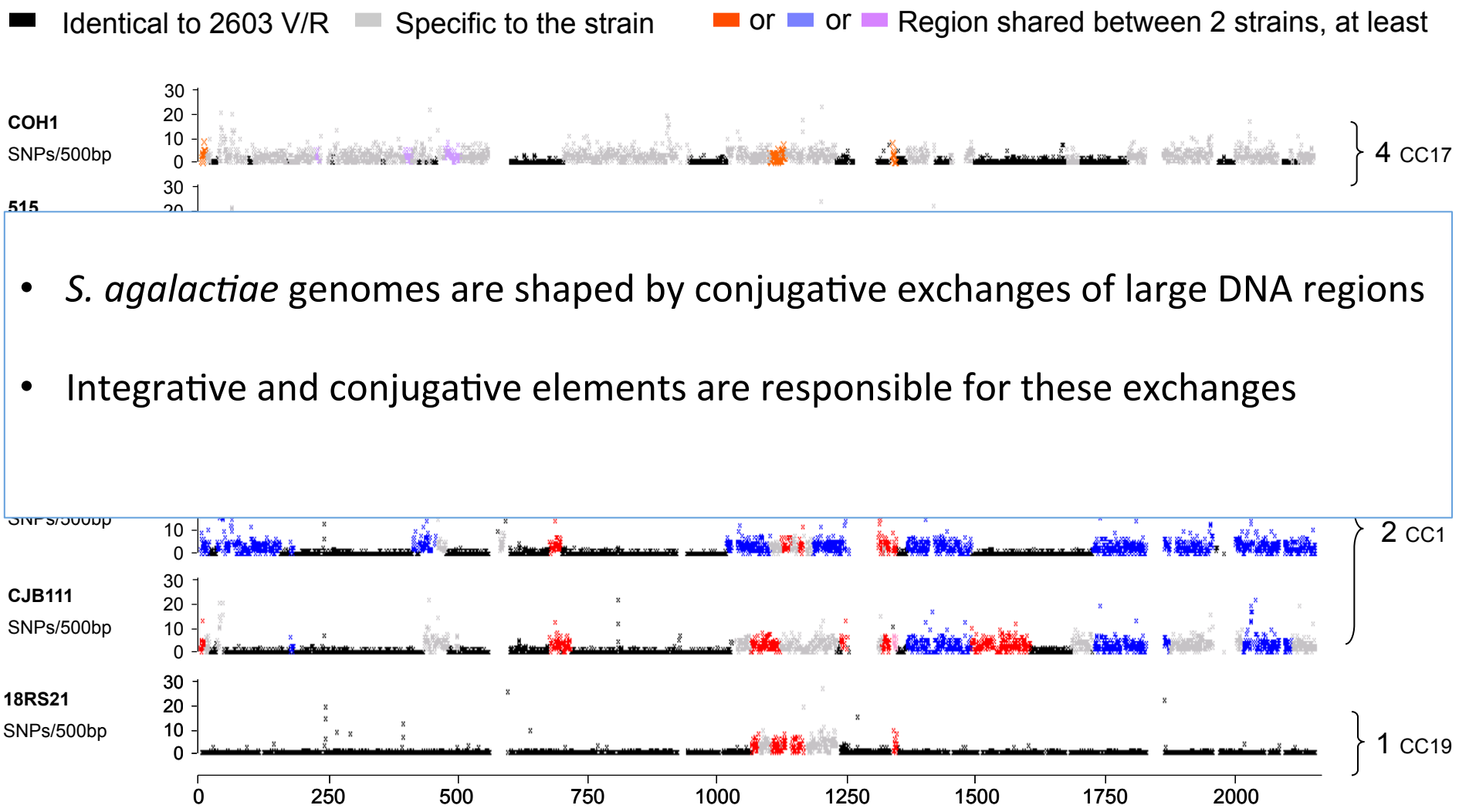
Tettelin *et al.* 2002

Polymorphisms among the 8 genome sequences



Brochet *et al.* 2008

Polymorphisms among the 8 genome sequences



- *S. agalactiae* genomes are shaped by conjugative exchanges of large DNA regions
- Integrative and conjugative elements are responsible for these exchanges

Brochet *et al.* 2008

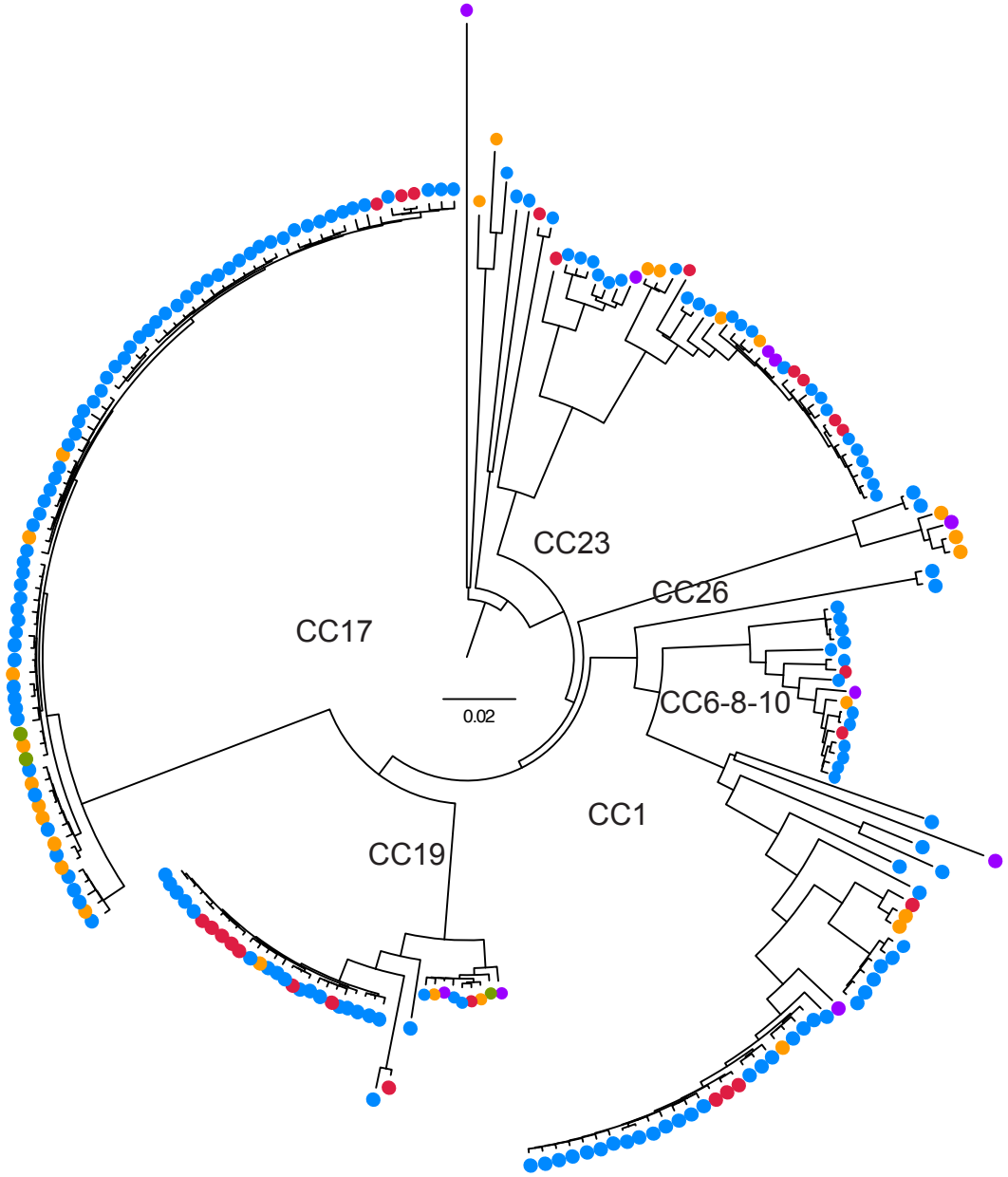
Population genomics of *S. agalactiae*

- Objective
  - To confirm the observed emergence of GBS infections at the bacterial population level
  - To get clues on the reasons for this emergence
- Strains:
  - Sanger Centre:
    - 92 European strains – DEVANI consortium (7 countries)
    - 24 Australian strains
  - Institut Pasteur:
    - 112 isolates: 27 from Africa, carriage and clinical isolates, 9 bovine strains.
    - 12 strains isolated between 1953 and 1961

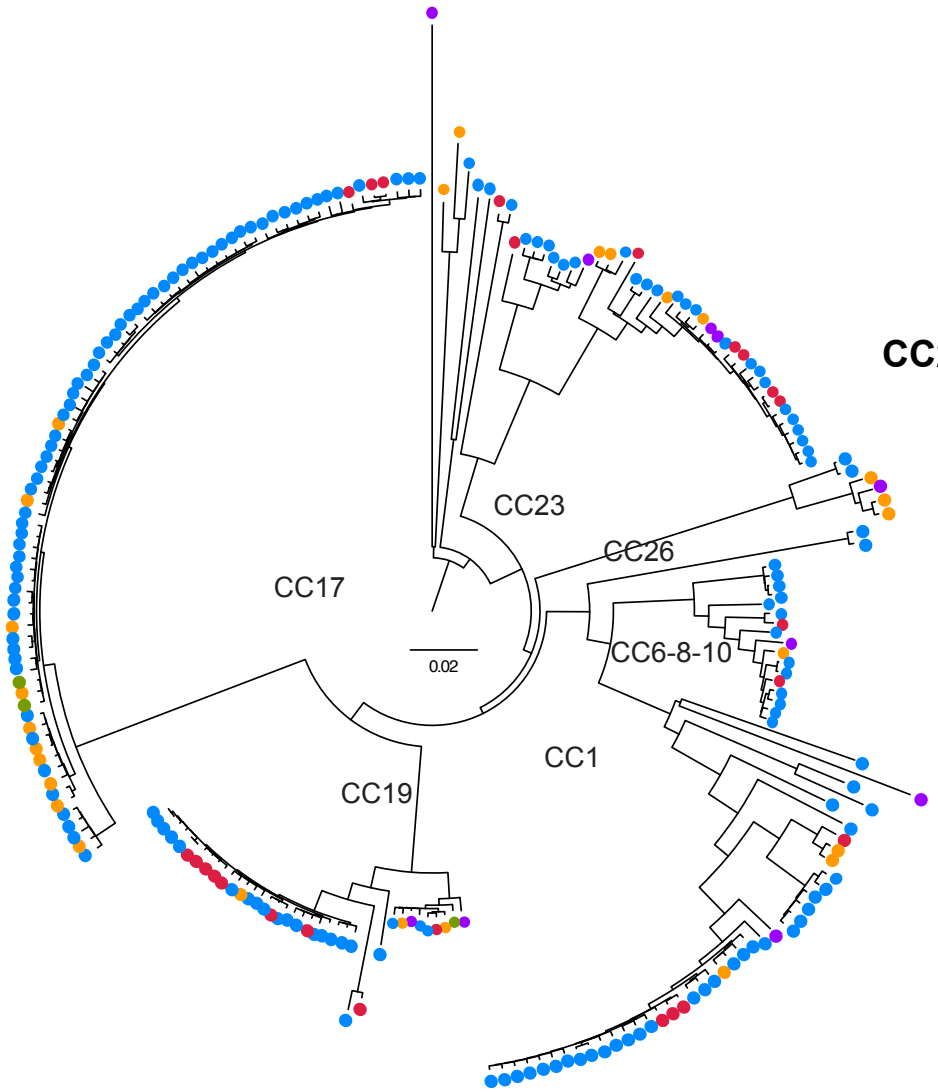
Methods

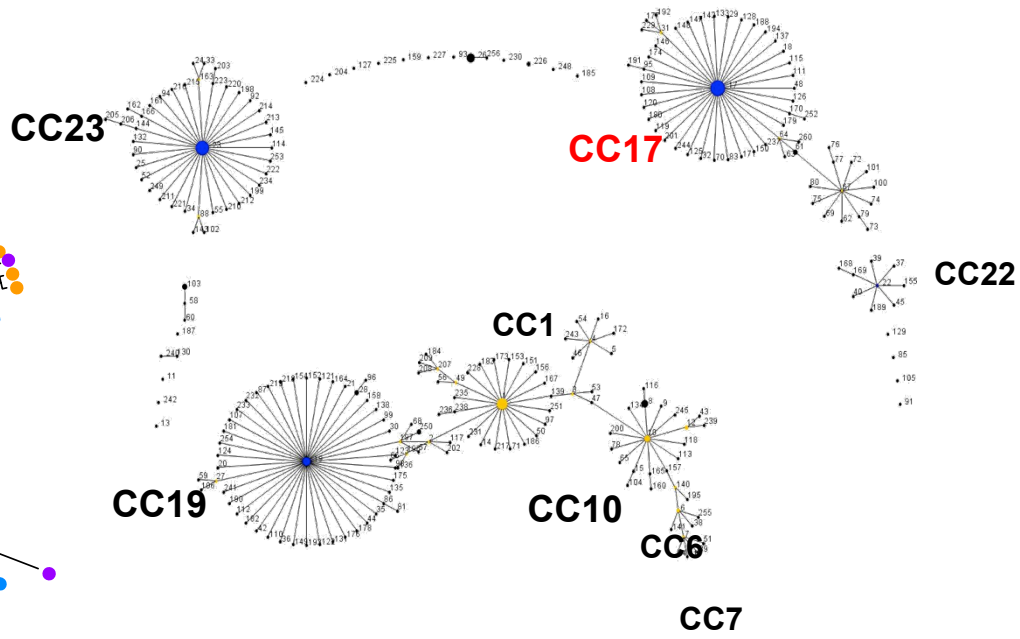
- Isolates were sequenced by the Illumina technology
- Assembled by using Velvet
- SNP were identified by using BWA and Mpileup (Samtools)
- Phylogeny was performed by maximum-likelihood and by using BEAST

Phylogenetic relationships between the 230 Isolates

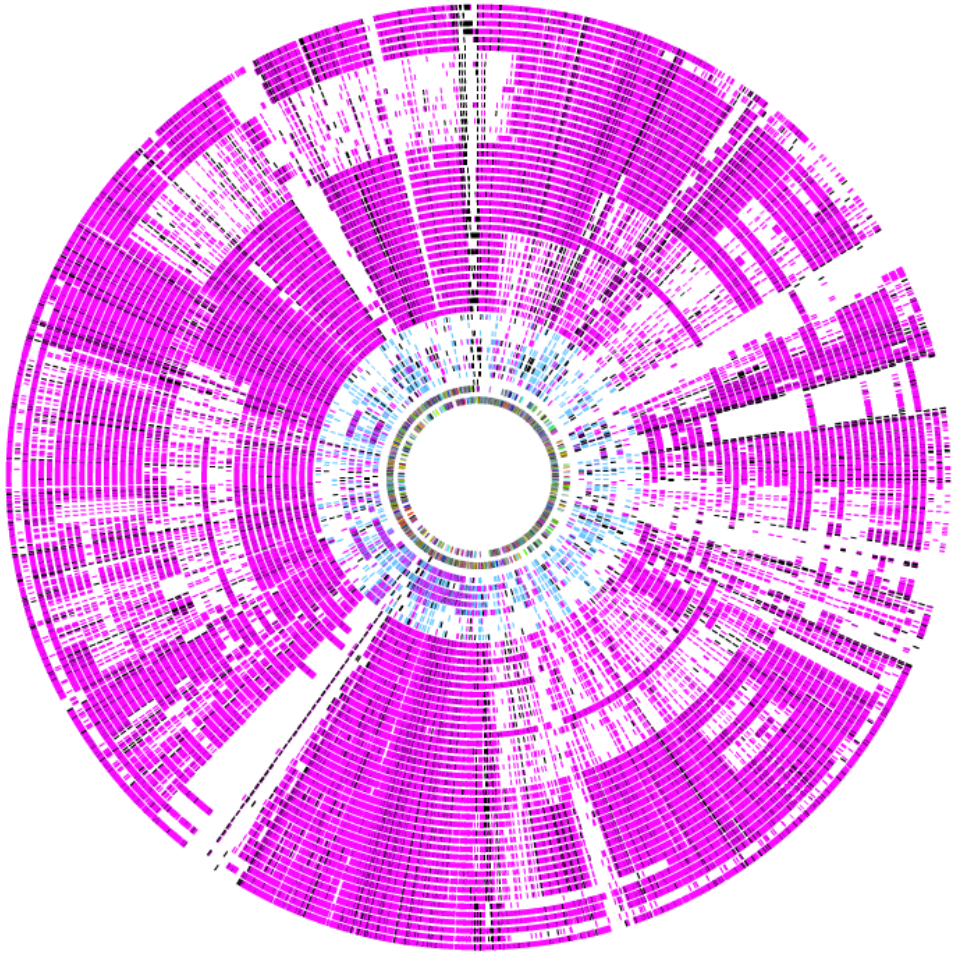


Whole genome sequence and MLST





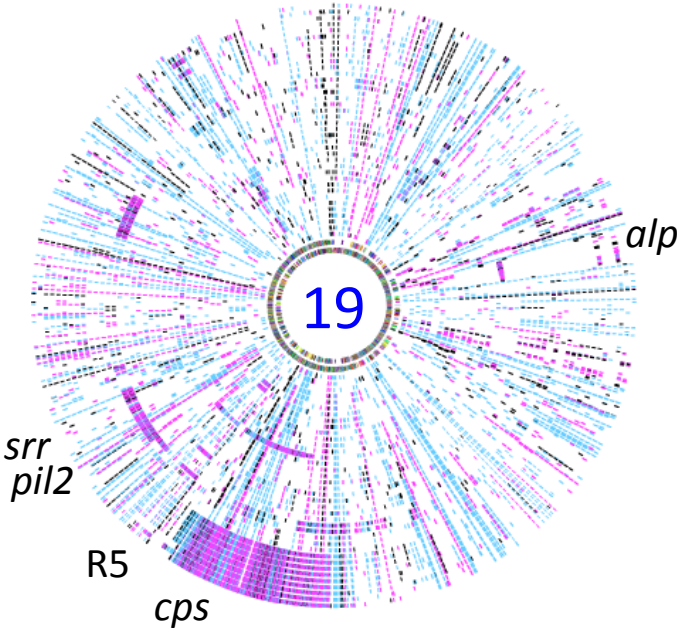
SNP distribution in 63 representative isolates

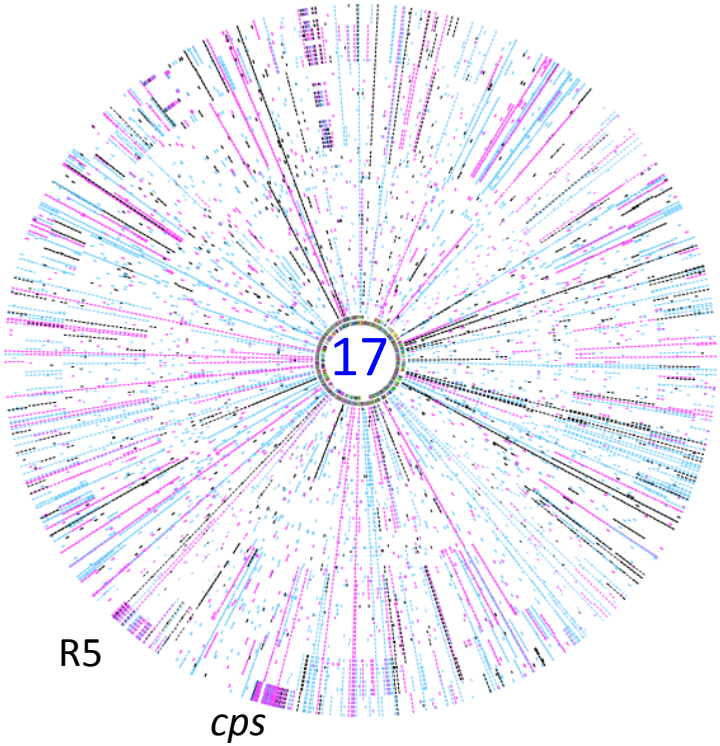


⇒ All parts of the genome have recombined

⇒ A true phylogeny of the species is not feasible

Recombination within CCs

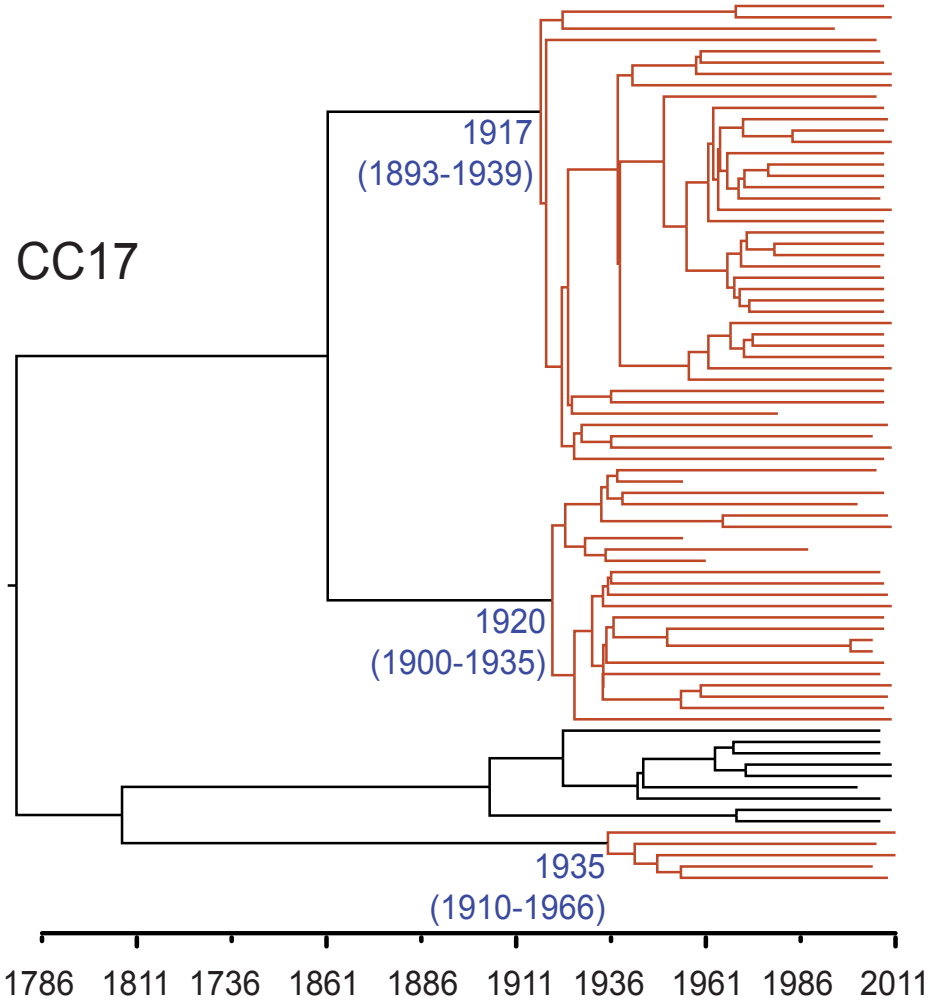




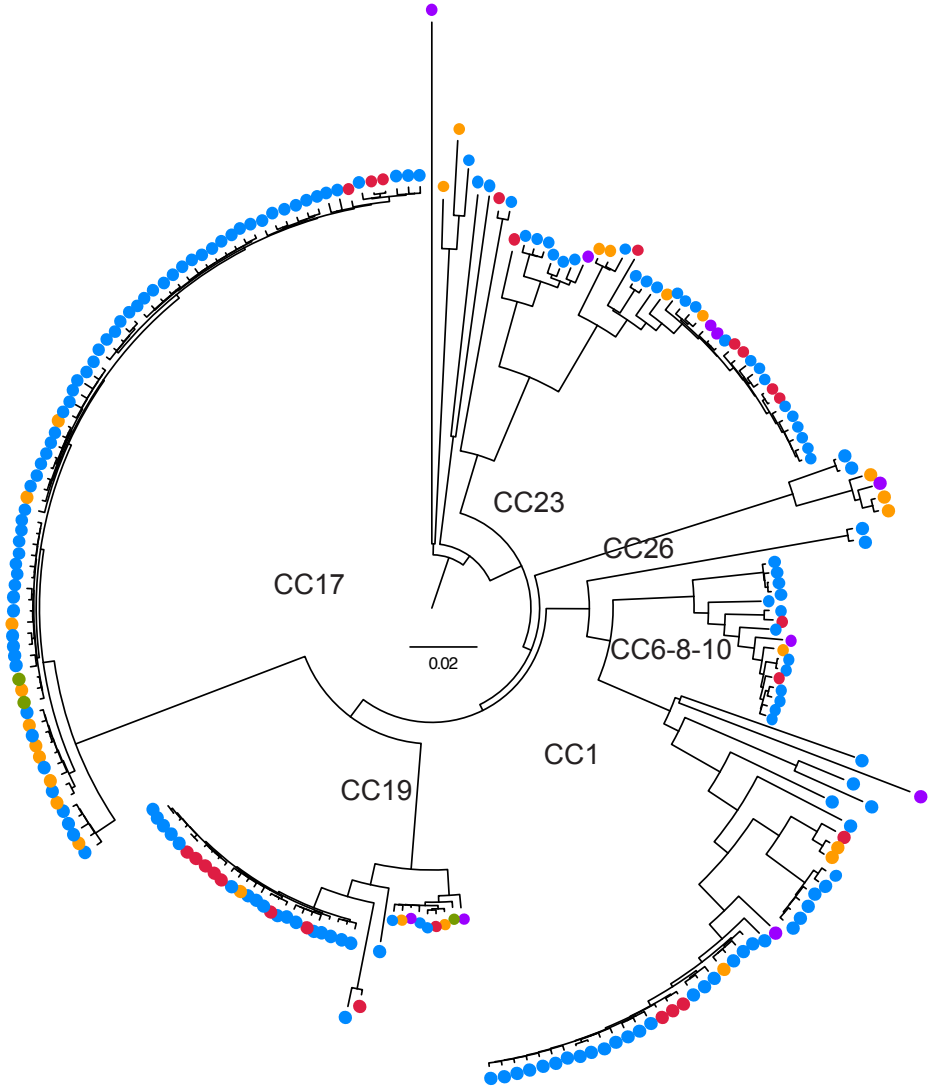
⇒ Recombination involves mostly regions encoding antigens

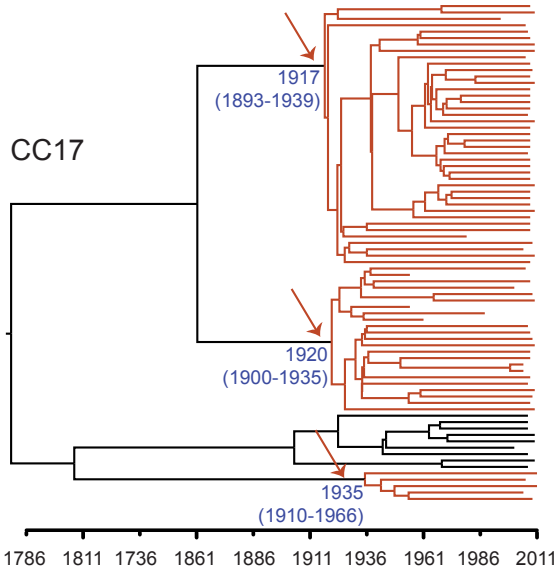
⇒ Low level of recombination among the hypervirulent CC17 strains

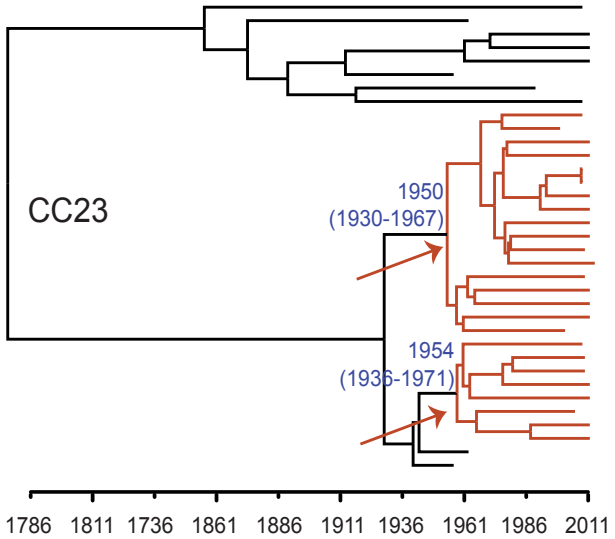
Time frame for the emergence of clones and complexes

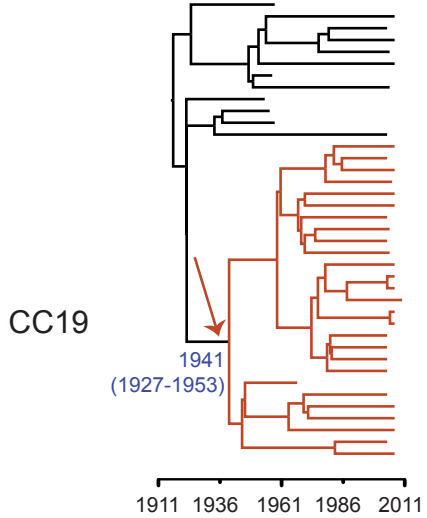


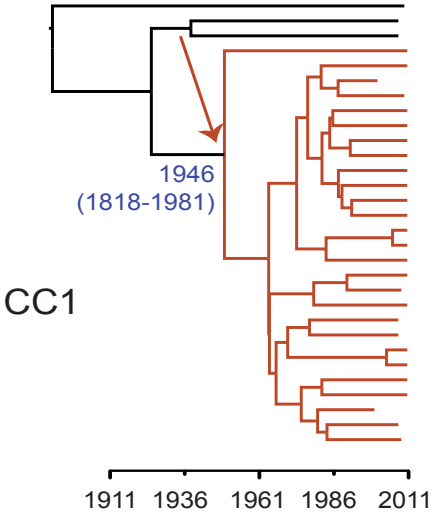
Bayesian analysis











- Similar timeframe for the origin of the 4 major clonal complex
- Timing for the emergence of major clones correspond to the emergence of GBS infections.

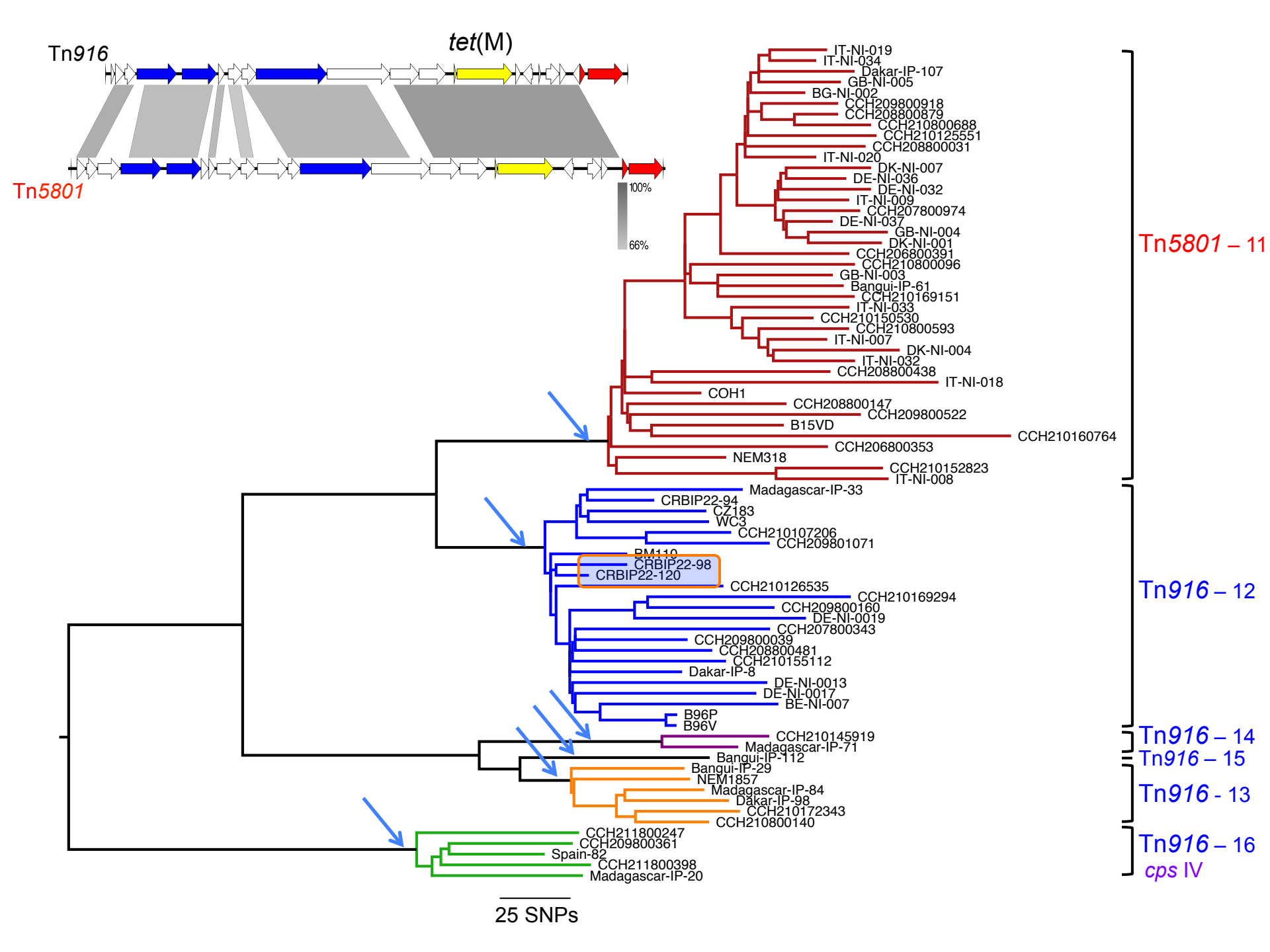
Antibiotic resistance in GBS

In all studies:

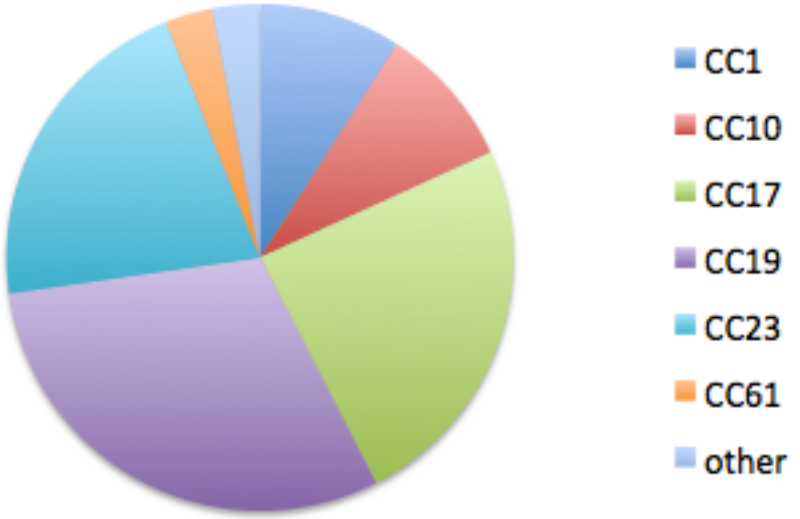
- More than 80 % of human isolates are tetracycline resistant

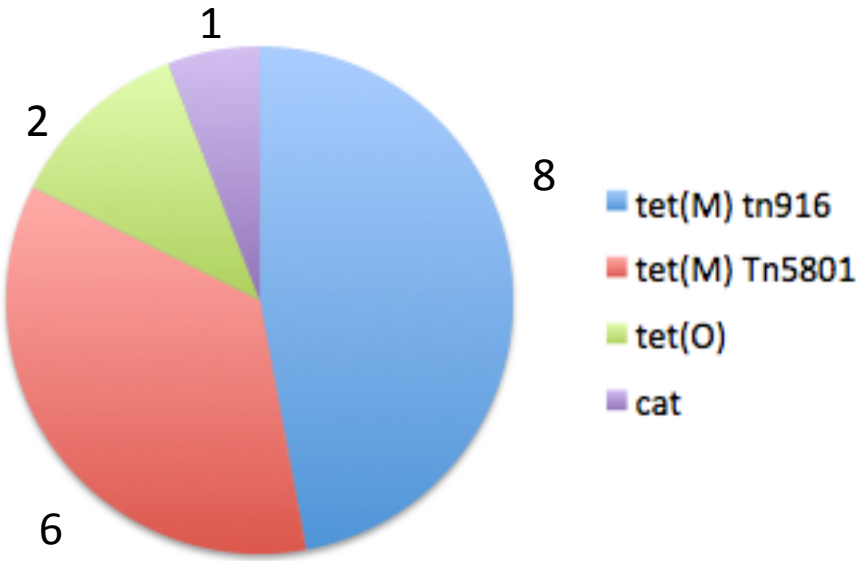
In the 230 analysed genomes

- 190 isolates express the *tet*(M) gene and 11 the *tet*(O) gene


Tn916
tet(M)
Tn5801
100%
66%
IT-NI-019
IT-NI-034
Dakar-IP-107
GB-NI-005
BG-NI-002
CCH209800918
CCH208800879
CCH210800688
CCH210125551
CCH208800031
IT-NI-020
DK-NI-007
DE-NI-036
DE-NI-032
IT-NI-009
CCH207800974
DE-NI-037
GB-NI-004
DK-NI-001
CCH206800391
CCH210800096
GB-NI-003
Bangui-IP-61
CCH210169151
IT-NI-033
CCH210150530
CCH210800593
IT-NI-007
DK-NI-004
IT-NI-032
CCH208800438
IT-NI-018
COH1
CCH208800147
CCH209800522
B15VD
CCH210160764
CCH206800353
NEM318
CCH210152823
IT-NI-008
Madagascar-IP-33
CRBIP22-94
CZ183
WC3
CCH210107206
CCH209801071
BM110
CRBIP22-98
CRBIP22-120
CCH210126535
CCH210169294
CCH209800160
DE-NI-0019
CCH207800343
CCH209800039
CCH208800481
CCH210155112
Dakar-IP-8
DE-NI-0013
DE-NI-0017
BE-NI-007
B96P
B96V
CCH210145919
Madagascar-IP-71
Bangui-IP-112
Bangui-IP-29
NEM1857
Madagascar-IP-84
Dakar-IP-98
CCH210172343
CCH210800140
CCH211800247
CCH209800361
Spain-82
CCH211800398
Madagascar-IP-20
25 SNPs
Tn5801 – 11
Tn916 – 12
Tn916 – 14
Tn916 – 15
Tn916 - 13
Tn916 – 16
cps IV

33 strains from the CIP isolated between 1953 and 1964





15 tetracycline R isolates

- ST distribution is not significantly different from today's distribution
- Tetracycline ressitance is present in half the isolates

Few facts

- Tetracycline a broad spectrum antibiotic extensively used starting in the 1950s
- The high rate of tetracycline resistance is specific to human strains compared to bovine strains (but not the use of tetracycline)
- Tetracycline is today rarely used but the tetracycline resistant remains at a high rate.

Proposed scenario for the emergence of GBS neonatal infections

Before 1950: a diverse population of GBS teracycline sensitive (unknown)

1950: Extensive use of tetracycline

1. Selection of TcR isolates by gain of mobile genetic element
2. Create a niche by eliminating TcS GBS and by altering the gut microbiota
3. Among TcR clones selection of those with higher colonization and dissemination properties
4. Worldwide dissemination of few TcR clones with higher virulence potential

(Da Cunha *et al*. Nature Communication 2014)

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C. Poyart
N. Dmytruk

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