



Genome-wide longitudinal analysis of *emm1* invasive Group A *Streptococcus* isolated from Belgian patients during 1994-2013

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Introduction

- **GAS**

Gram-positive bacterial pathogen

Causes human infections worldwide

Diverse clinical manifestations

Pharyngitis

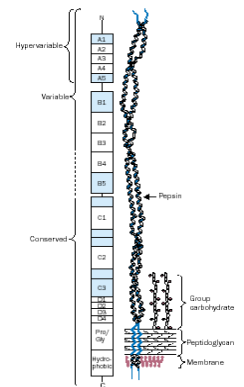
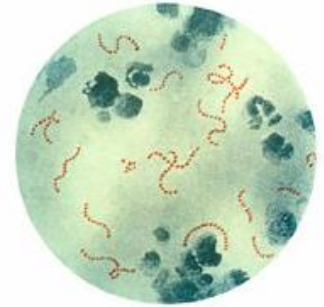
Invasive infections

Streptococcal toxic shock syndrome

- ***Emm1* gene**

Encode for M protein

Antiphagocytic cell surface molecule



Aim of the study

Using whole genome mapping and whole genome sequencing to longitudinal study genome wide evolutionary events in *emm1* GAS strains collected in Belgium

- 1994-2013
- invasive strains
- non-invasive strains

Whole genome mapping (WGM)

WGM is an ordered, high-resolution density whole genome restriction map generated from single DNA molecule

Creating a whole genome map:

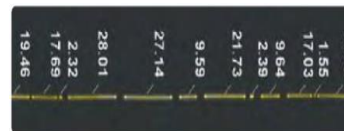
DNA
extraction



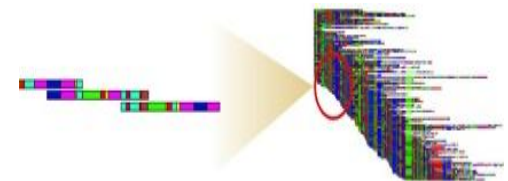
DNA
molecules
immobilized on
flow chip



RE digest
fluorescent
stain

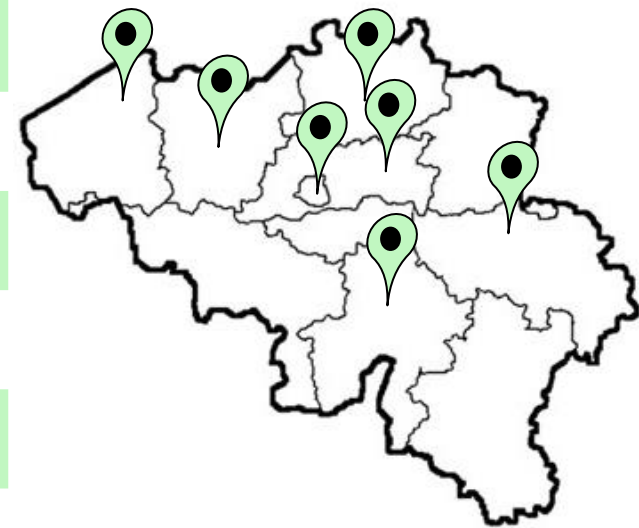


Analysis

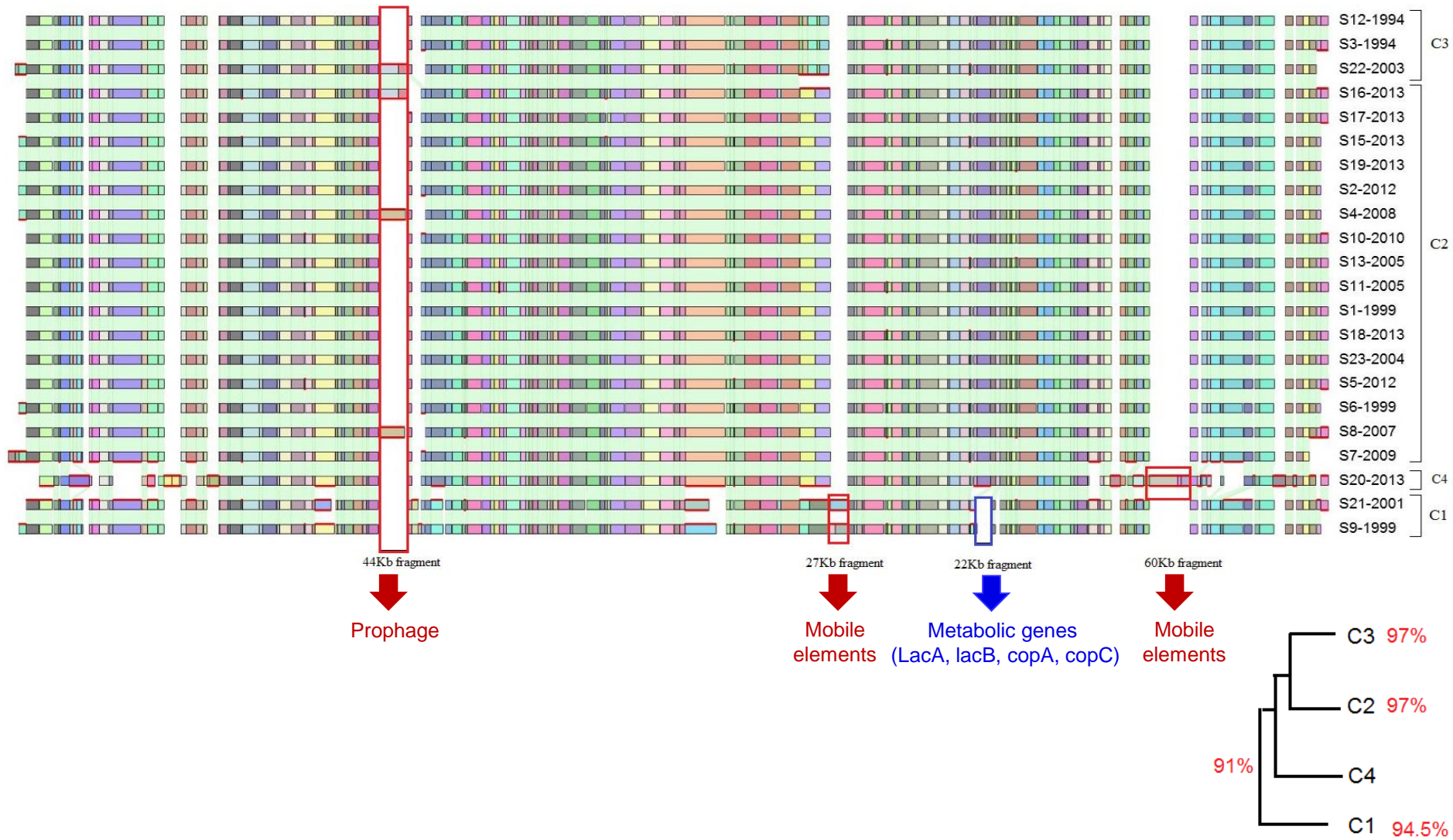


Longitudinal collection of *emm1* GAS

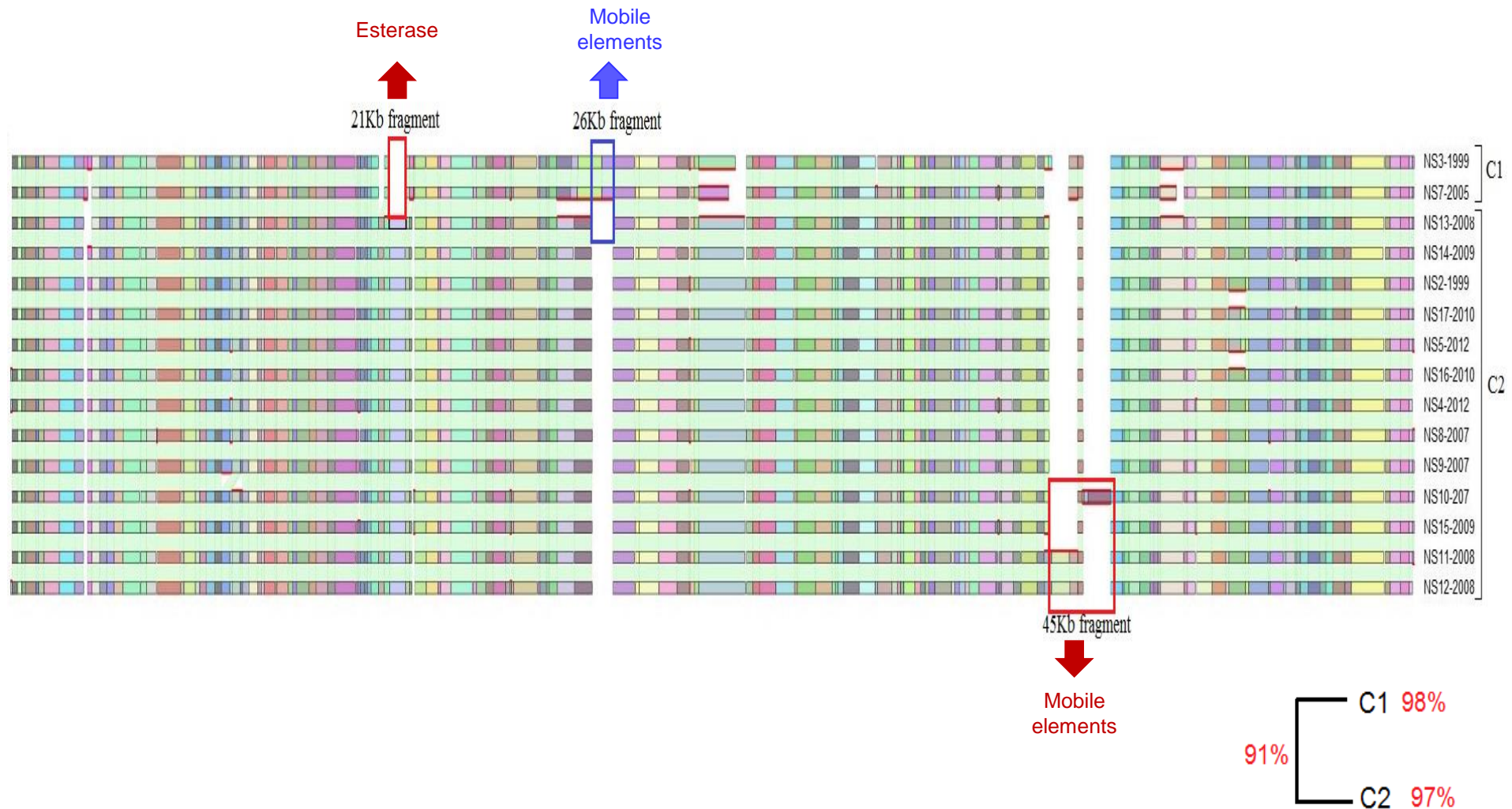
Year	Strains	Invasive	Source	MLST	PFGE	WGS
1994	2	Yes	ND	ST-28	03a	Yes
1999	3	Yes	Blood	ST-28	03a	Yes
	2	No	Throat	ND	<i>sfil</i>	No
2001	1	Yes	Blood	ST-28	03a	Yes
2003	1	Yes	Blood	ST-28	03a	Yes
2004	1	Yes	Blood	ST-28	03a	Yes
2005	2	Yes	Blood	ST-28	03a	Yes
	2	No	Throat	ND	<i>sfil</i>	No
2007	1	Yes	Tissue	ST-28	03f	Yes
	3	No	Throat	ND	03a	Yes
2008	1	Yes	Blood	ST-28	03f	Yes
	3	No	Throat	ND	03a	Yes
2009	1	Yes	Blood	ST-28	03a	Yes
	2	No	Throat	ND	03a	No
2010	1	Yes	wound, not sterile	ST-28	03a	Yes
	2	No	Throat	ND	03a	No
2012	2	Yes	Blood (1), Tissue (1)	ST-28	03a	Yes
	2	No	Throat	ND	03a	No
2013	5	Yes	Other invasive (3); blood (2)	ST-28	03a	Yes
	3	No	Throat	ND	03a	No



Whole genome maps of *emm1* invasive strains (1994-2013)



Whole genome maps of *emm1* non-invasive strains (1994-2013)



Whole genome sequencing of invasive *emm1* GAS

- MiSeq Illumina
- *De novo* Genome assembly (Velvet & SPAdes)
- Annotation (RAST Server <http://rast.nmpdr.org/>)
- Comparative genome analysis (MUMmer and mauve)
- Prophages, crispr

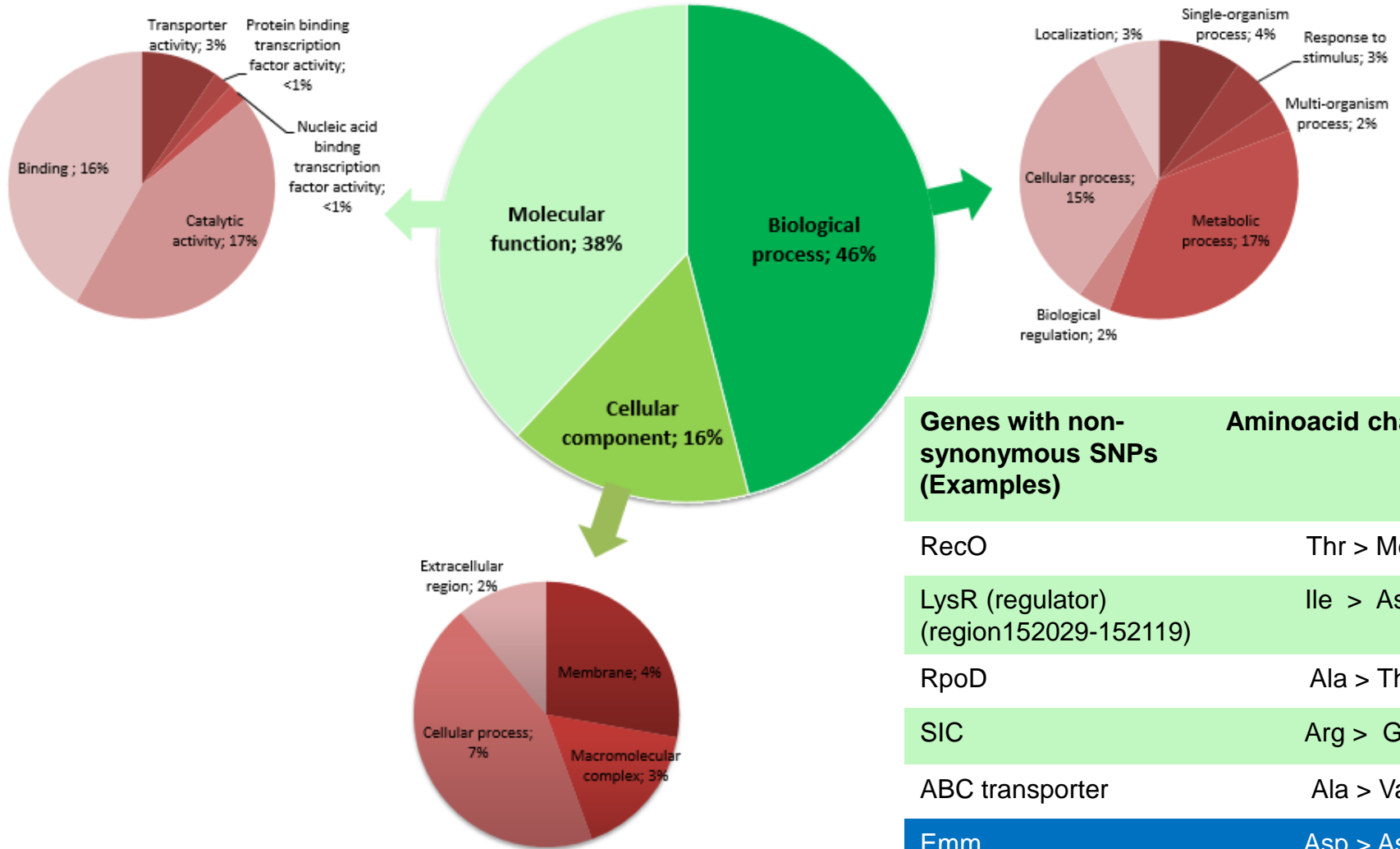


Average genome size (bp): ~1865579

Average GC content (%): ~38

Non-synonymous SNPs (longitudinaal)

SNP: Single-nucleotide polymorphism



36 Kb Recombination spot

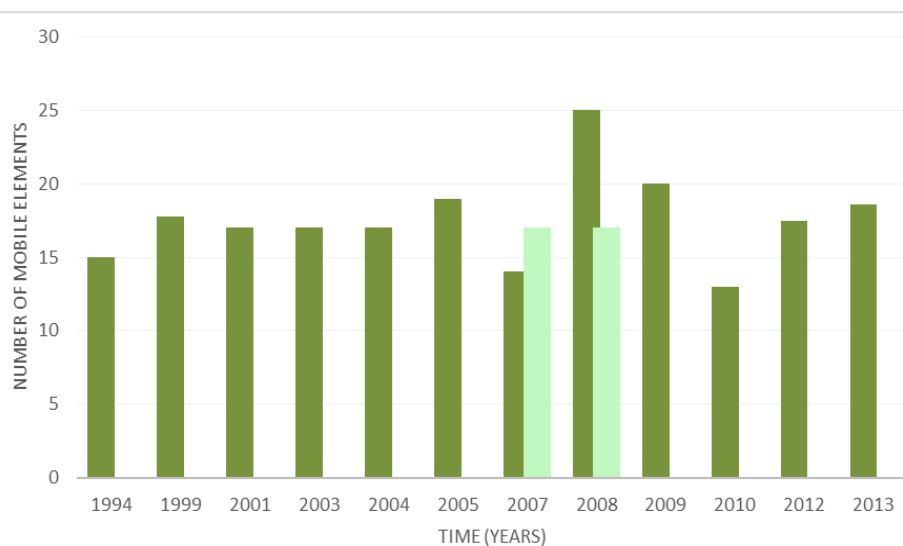


36 kb with high number of mutation (*purA**nadC*)

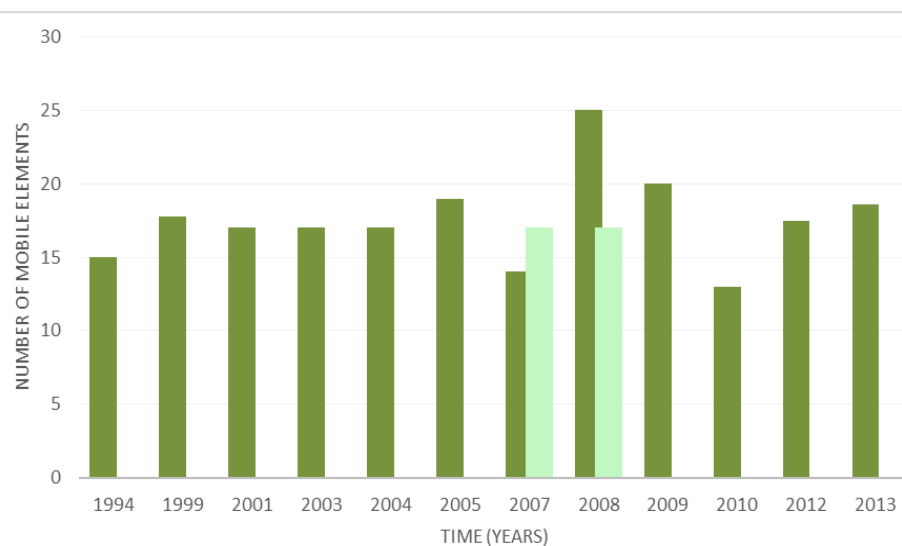
Comparison of core genome versus reference	SNPs	Non-synonymous SNPs
1994 versus MGAS5005	94	32
1994 versus 2013	82	37
2013 versus MGAS5005	1	1
2007 invasive versus MGAS5005	8	2
2008 non-invasive versus MGAS5005	70	37

Prophages and other mobile elements in invasive *emm1* GAS during 1994-2013

Prophages



Mobile elements (transposons)



■ Invasive GAS strains
■ Non-invasive GAS strains

Prophages presence in *emm1* GAS

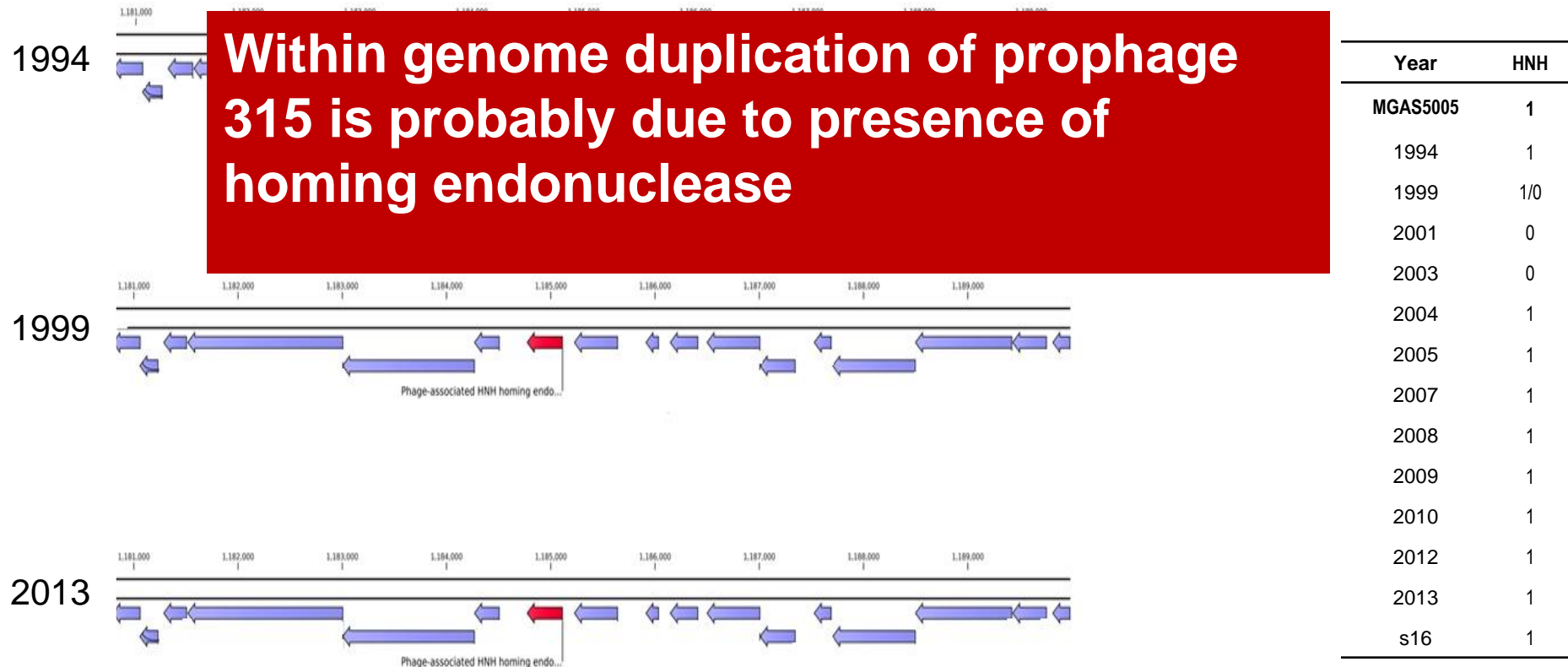
Invasive strains	1994 (n=1)	1999 (n=2)	2001 (n=1)	2003 (n=1)	2004 (n=1)	2005 (n=2)	2007 (n=1)	2008 (n=1)	2009 (n=1)	2010 (n=1)	2012 (n=1)	2013 (n=6)
PHAGE_Strept_pyogenes_315_5	2	2+3	1 (incomplete)	1	1	2+3	3	3	2	3	2	2-4
PHAGE_Strept_P9	1	1+1	0	1	1	1	1	1	1	1	1	1-2
PHAGE_Enterococcus_G4_sensu_lato	0	0	0	0	0	0	0	0	0	0	1 (S2)	0
PHAGE_Strept_IC1	0	0	0	0	0	0	1	0	0	0	0	0
PHAGE_Temper_phiNIH1_1	0	0	0	0	0	0	0	0	0	0	0	1 (S16)
PHAGE_Strept_PH10	0	1	0	0	0	0	0	0	0	0	0	0

→ HNH-Homing endonucleases

Non-invasive strains	2007 (n=1)	2008 (n=1)
PHAGE_Strept_pyogenes_315_5	1	1
PHAGE_Strept_P9	1	1

Gain of intron containing HNH-Homing endonucleases in phage 315

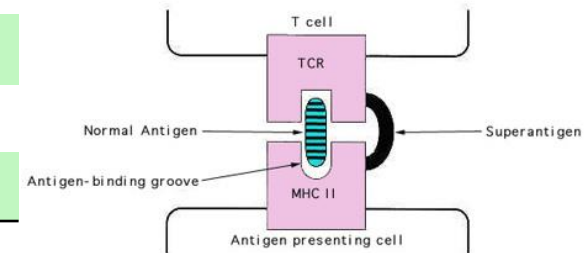
Endonucleases encoded by introns promote the homing of their respective genetic elements into allelic intronless site.



Superantigens distribution in invasive GAS

Year	Superantigens						
	SpeG	SpeA	SpeB	SpeK	SmeZ	SpeJ	SpeJ-fragment
1994	1	1	1	0	0	1	0
1999	1	1	1	0	0	1	1
2001							
2003							
2004							
2005							
2007	1	1	1	1	1	1	0
2008	1	1	1	1	1	1	0
2009	1	1	1	0	0	1	0
2010	1	1	1	0	0	1	1
2012	1	1	1	0	0	1	0
2013	1	1	1	0	0	1	0
s16	1	1	1	1	1	1	0

SpeJ might be phage associated and thus transferable between GAS

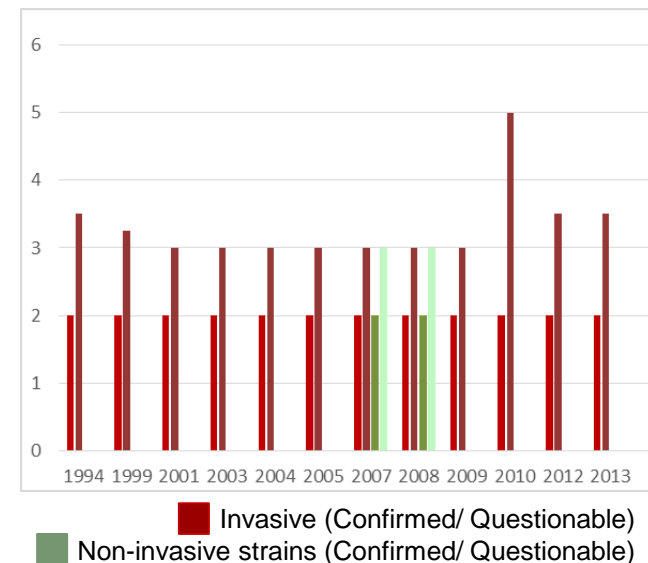


Correlation of CRISPR and Prophages

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats provide acquired immunity against foreign genetic elements

There is no correlation between CRISPR/CAS genes and the gain/ loss of prophages

Strain	CRISPR/CAS	Prophages	Source
SF-370	2 (3)	3	NC_007297.1
MGAS-5005	2 (3)	3	NC_007297.1
M1-476	2 (3)	3	NC_020540.2
S3-1994	2 (4)	3	This study
S1-1999	2 (3)	3	This study
S8-2007	2 (3)	4	This study
S4-2008	2 (3)	4	This study
S16-2013	2 (4)	5	This study



Conclusion

- Longitudinal genome wide analysis showed a rather **high conservation** of the *emm1* GAS strains in Belgium during the 20-year period studied
- Gain/presence of **HNH-Homing Endonuclease** in phage 315 linked to duplication
- Phage associations of **SpeJ**
- No correlation between **CRISPRs** and prophages
- Variations in **2007 and 2008** might be link to specific selection pressure
- **WGM** is a high resolution technique for detection of genomic variations, compared to standard molecular typing methods, as MLST, PFGE.

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Thank you for your attention