

The mobilome; a major contributor to *Escherichia coli* *stx2*-positive O26:H11 strains intra-serotype diversity

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Introduction

- Several clones of STEC O26:H11/H- with distinct genetic characteristics have been identified and characterized.
- A highly virulent clone possessing the *stx2* gene has emerged in the last decade.

Objective : Gain a better insight into the phylogenetic relationships of the various *stx2*-positive O26:H11 strains circulating in France.

Methods

- WGS (Illumina + PacBio) was performed on 8 French clinical *stx2*-positive O26:H11 strains.
- Comparative and phylogenetic analysis of these strains with 10 other O26:H11/H- genomes obtained from publicly available databases.

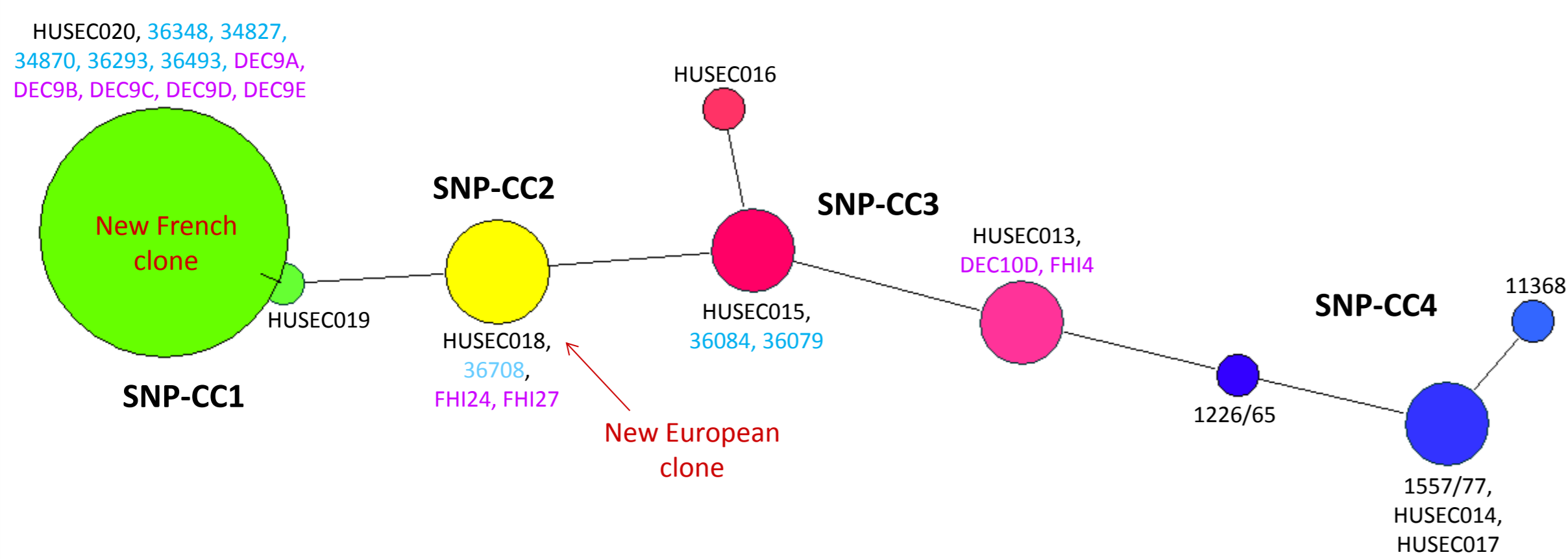
Genomes used in this study

Designation	Sample	Stx subtype	MLST	SNP-CC	SP_O26-E	Country of isolation (year)	Symptoms	Accession number
EHEC O26:H11	11368	1a	ST21	CC4	-	Japan (2001)	Diarrhea	NC_013361.1
	36084	2a	ST21	CC3	-	France (2013)	HUS	LDX000000000
	36079	2a, 2a	ST21	CC3	-	France (2013)	HUS	LDX000000000
	FHI4	2a	ST21	CC3	-	Norway (2002)	HUS	GCF_000951835.1
New European clone (Bielaszewska et al. 2013)	36708	2a	ST29	CC2	-	France (2013)	HUS	LDXG000000000
	FHI24	2a	ST29	CC2	-	Norway (2007)	HUS	GCA_000936225.1
	FHI27	2a	ST29	CC2	-	Norway (2008)	HUS	GCA_000951875.1
	34827	2a, 2a	ST29	CC1	+	France (2012)	HUS	LDXF000000000
New French clone (Delannoy et al. 2015)	34870	2a	ST29	CC1	+	France (2012)	HUS	LDXE000000000
	36348	2d	ST29	CC1	+	France (2013)	HUS	LDXD000000000
	36293	2d, 2d	ST29	CC1	+	France (2013)	HUS	LDXC000000000
	36493	2d	ST29	CC1	+	France (2013)	HUS	LDXB000000000
AEEC O26:H11	DEC10D	-	ST21	CC3	-	France (1952)	Diarrhea	NZ_AIGS000000000.1
	DEC9A	-	ST29	CC1	-	USA (1961)	Diarrhea	NZ_AIGK000000000.1
	DEC9B	-	ST29	CC1	+	USA (1979)	Diarrhea	NZ_AIGL000000000.1
	DEC9C	-	ST29	CC1	+	Switzerland (1952)	Diarrhea	NZ_AIGM000000000.1
	DEC9D	-	ST29	CC1	+	Denmark (1967)	Diarrhea	NZ_AIGN000000000.1
	DEC9E	-	ST29	CC1	+	Mexico (1986)	Diarrhea	NZ_AIGO000000000.1
	DEC9F	-	ST29	CC1	+	Mexico (1986)	Diarrhea	NZ_AIGO000000000.1

SNP typing of the isolates

48 informative SNPs cluster EHEC O26:H11 in 4 clonal complexes (CC):

- The new European clone belongs to SNP-CC2
- The new French clone belongs to SNP-CC1

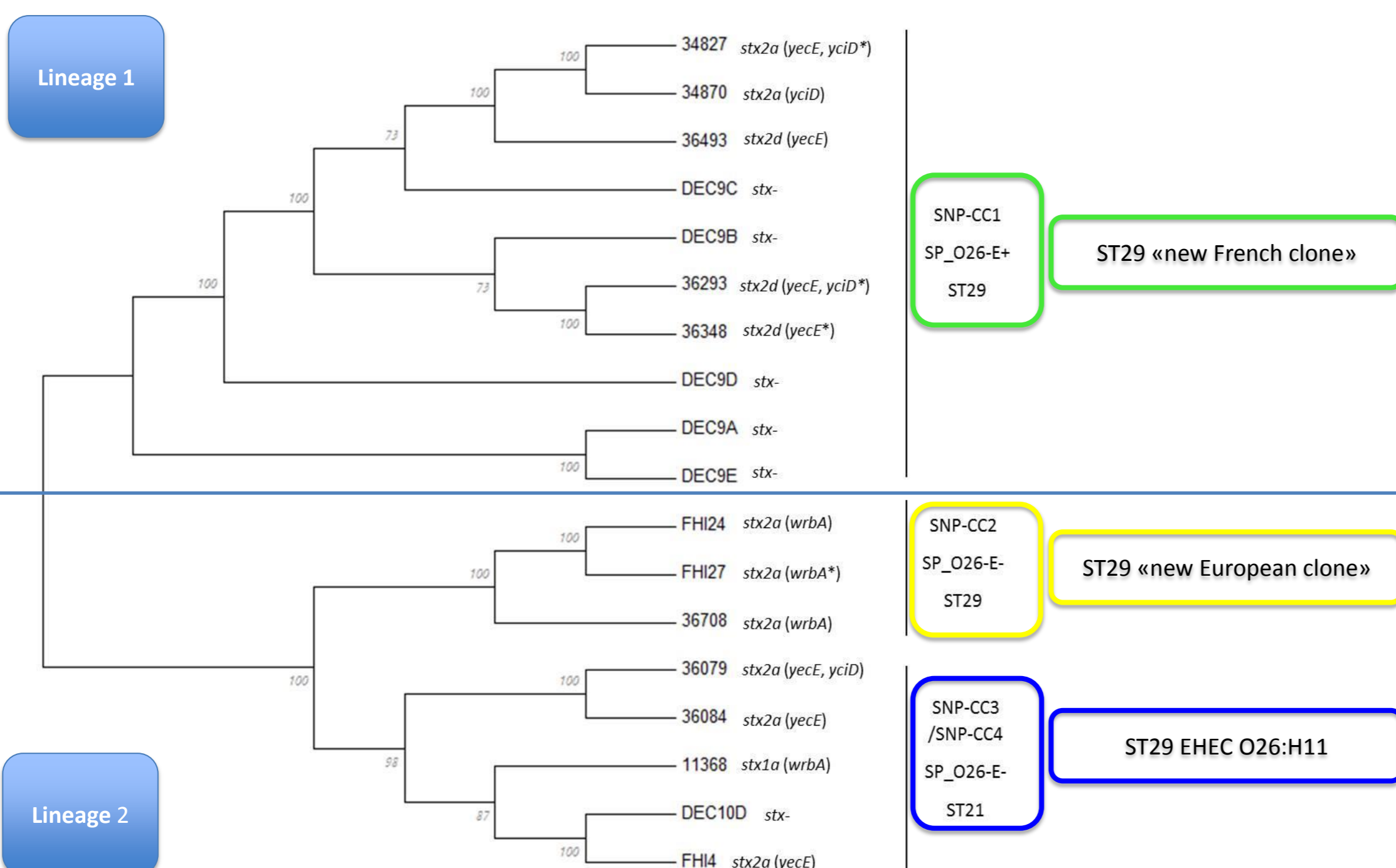


Minimum spanning tree based on 48 SNPs.

Whole genome SNPs

2 lineages :

- Lineage 1 = ST29 “new French clone” (SNP-CC1).
- Lineage 2 = ST21 strains (SNP-CC3 and SNP-CC4) and ST29 “new European clone” (SNP-CC2)

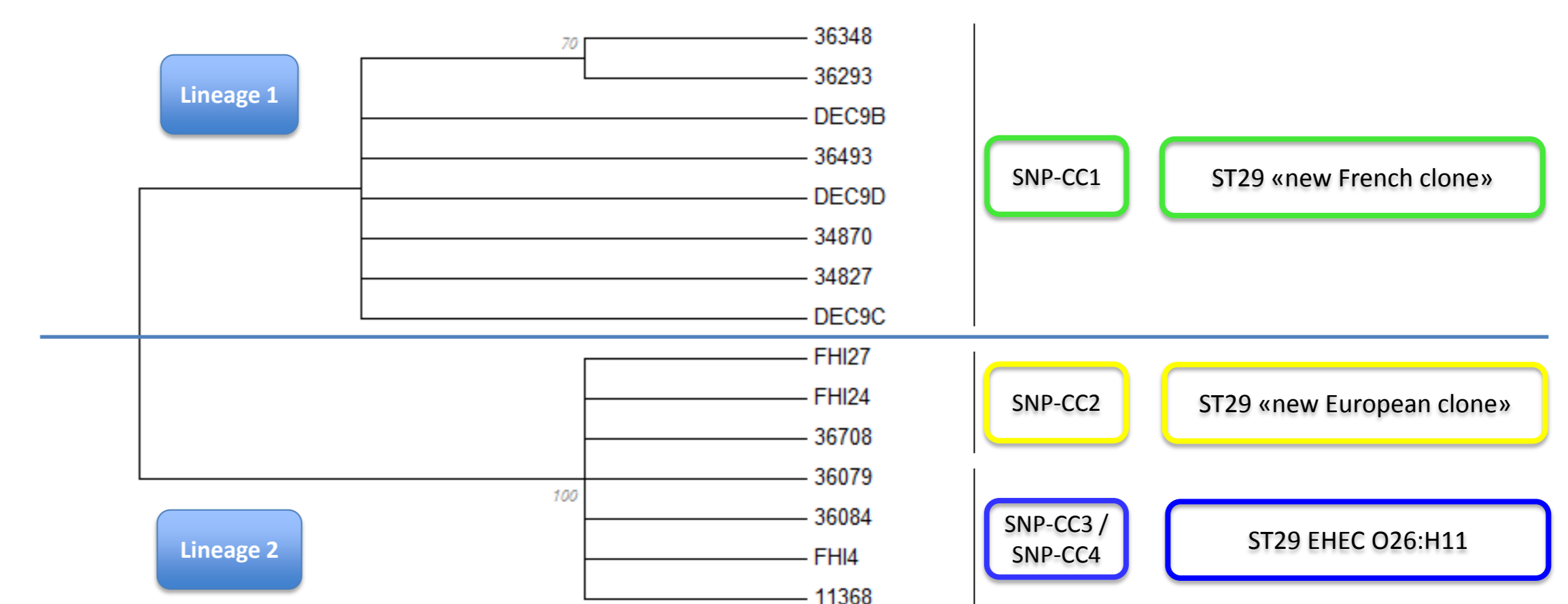


Maximum likelihood tree based on wgSNPs.

CRISPR array and cas genes comparison

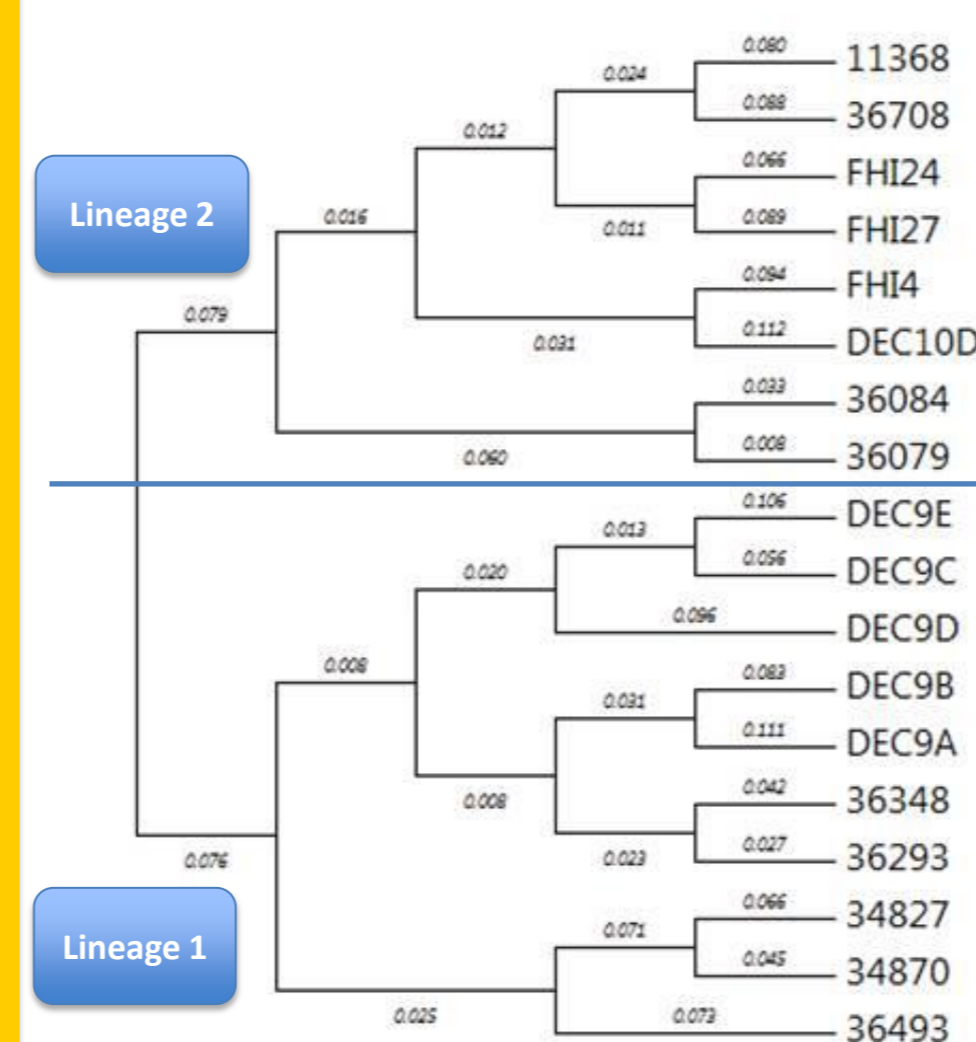
Global congruence between the CRISPR array and the phylogenetic background

A simple qPCR assay targeting the CRISPR array specific to SNP-CC1 (SP_O26-E) can distinguish between the 2 main lineages.



Maximum likelihood tree based on the concatenated ORFs of the 8 cas genes.

Analysis of the Mobile Genetic Elements (MGEs) of the strains



Phylogenetic relationships of the phageome of O26:H11/H- strains

- Numerous MGEs (phages + plasmids) in each strain : 8.7–19.8% of the total genome size.

- Complex history of recombination

- Each clonal complex (SNP-CC) is characterized by a unique set of plasmids and phages, including *stx*-prophages, suggesting evolution through separate acquisition events.

- Phylogeny inferred from phageome comparison clusters the strains in the same groups as core genome analysis and wgSNPs

Continuing evolution of EHEC O26:H11

- Different clones from 2 lineages are circulating in France, including the new, highly pathogenic, European EHEC O26:H11 clone (*stx2*+, ST29, CC2) and the new ‘French’ clone (*stx2*+, ST29, CC1)
- The PCR test SP_O26-E could be a useful addition for rapid investigation of the new French O26:H11 clone.
- There is a close phylogenetic proximity of the “new French clone” to the non-STEC or AEEC O26:H11 strains :
 - ⇒ direct evolution of this clone from an AEEC clone with uptake of *stx*-phages ?
- Overall, the MGEs appear to play a major role in O26:H11 intra-serotype clonal diversification.