



## Occurrence of IS6110 copies in genomes of field strains of *Mycobacterium bovis* revealed high disparity among genetic family

Ciriac Charles, Lorraine Michelet, Cyril Conde, Franck Biet, Maria-laura Boschioli

### ► To cite this version:

Ciriac Charles, Lorraine Michelet, Cyril Conde, Franck Biet, Maria-laura Boschioli. Occurrence of IS6110 copies in genomes of field strains of *Mycobacterium bovis* revealed high disparity among genetic family. 7th International conference on *Mycobacterium bovis*, Jun 2022, Galway, Ireland. anses-03947254

**HAL Id: anses-03947254**

**<https://anses.hal.science/anses-03947254>**

Submitted on 19 Jan 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



# OCCURRENCE OF IS6110 COPIES IN GENOMES OF FIELD STRAINS OF *MYCOBACTERIUM BOVIS*

## REVEALED HIGH DISPARITY AMONG GENETIC FAMILY

CHARLES Ciriac<sup>1,2</sup>, MICHELET Lorraine<sup>1</sup>, CONDE Cyril<sup>2</sup>, BIET Franck<sup>2</sup> and BOSCHIROLI Maria-Laura<sup>1</sup>

<sup>1</sup> Anses Maisons-Alfort, Unité des Zoonoses Bactériennes. <sup>2</sup> INRAE Nouzilly Infectiologie et Santé Publique. / Contact: [ciriac.charles@anses.fr](mailto:ciriac.charles@anses.fr)

IS6110 is an insertion sequence found in the *Mycobacterium tuberculosis* complex (MTBC), with an important role in genome plasticity and in bacterium evolution. Within the MTBC, *Mycobacterium bovis* (*M. bovis*) had largely been considered as possessing one or very few copies of IS6110 (1). However, we showed by complete genome sequencing, that *M. bovis* Mb3601 (2), a strain with a widespread genotype from the bTB endemic region of Côte d'Or (Figure 1), possesses eleven copies of IS6110.

### GOAL OF THE STUDY

We therefore studied if i) other French *M. bovis* strains present this IS6110 high-copy number trait and ii) there is any evolution of the number and location of IS6110 in the genomes over time and depending on the infected animal species on sympatric strains of Dordogne Haute-Vienne, the French region with more bTB outbreaks, with a specific genotype, SB0120-DHV.

### MATERIALS AND METHODS

ISMMapper (version 2.0.1) (3) was used for identification of IS6110 on Illumina genomes using Mb3601 as reference on:

- Panel 1: **80 *M. bovis*** genomes of French representative genotypes (4) (Figure 1).
- Panel 2: **227 *M. bovis*** SB0120-DHV sympatric strains issued from wild and domestic animals (183 cattle, 26 badger, 12 wildboar, 3 fox, 2 deer and 1 roe deer) between 2001 and 2017 (Figure 2).

### IS6110 IN THE *M. BOVIS* FRENCH DIVERSITY

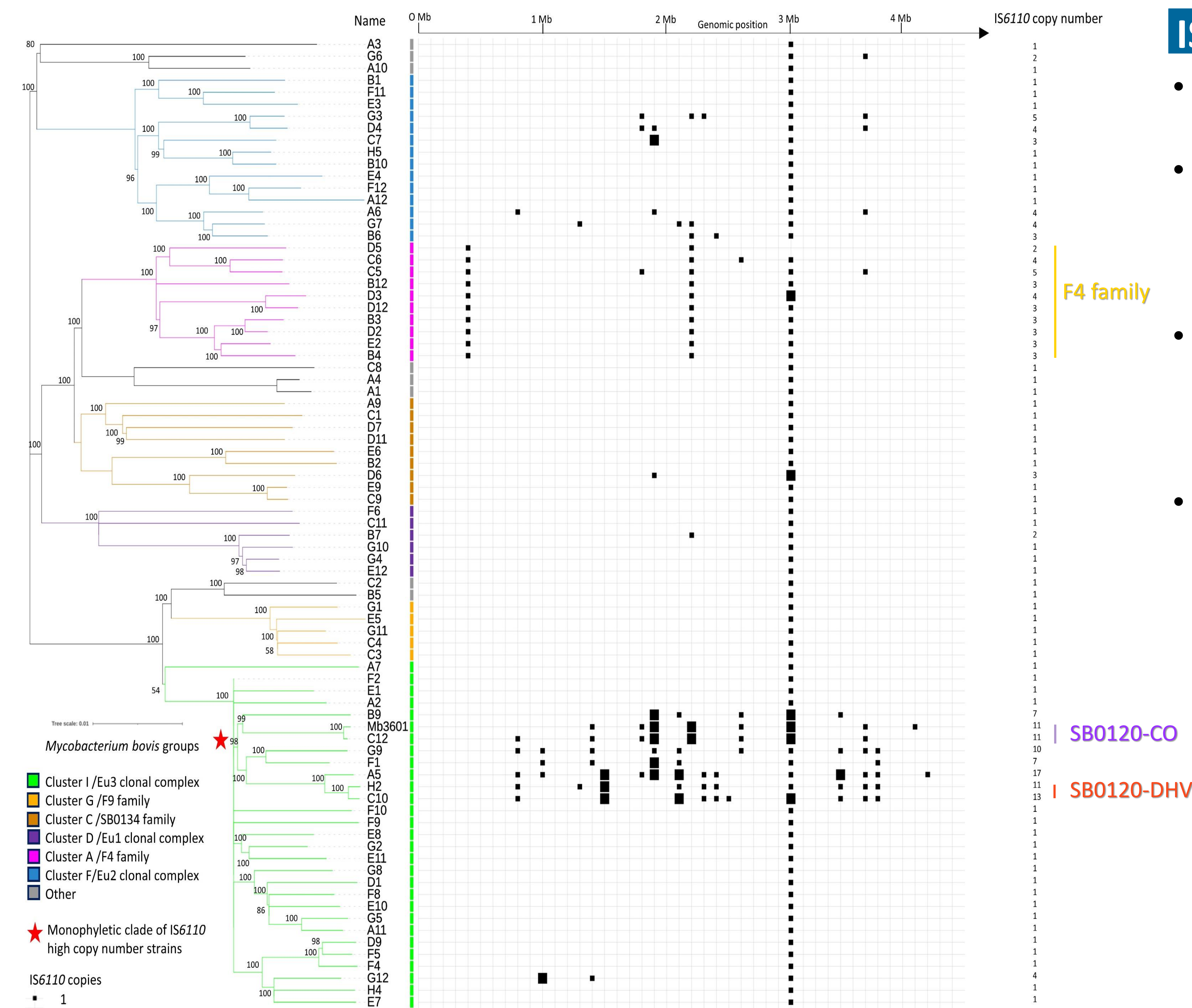
- A third of the strains in our sample are **IS6110 multi-copy**; 10% present more than 6 copies (Figure 1).
- These strains with the **highest IS6110 copy numbers** are those circulating in the **most bTB affected regions** like Côte d'Or (SB0120-CO) or Dordogne Haute-Vienne (SB0120-DHV) in last years (5-6) and are defined by a **monophyletic clade** in the tree (Figure 1).
- IS6110 copy numbers also correlates with clonal group definition, as several genomes of **the same clade present the same or very similar IS6110 copy numbers** (the F4 family and the previously mentioned groups) (Figure 1).
- **58 insertion sites** were identified on Panel 1. Five of them are shared by 5 or more strains; they are either specific to a clonal group (insertion site 1) or shared by several clonal groups (Table 1). Some genes interrupted by IS6110 could play a role in bacteria phenotype.

**Table 1:** Representation of IS6110 site with their gene environment. Genomic positions were deduced through ISMapper analysis. Surrounding genes of these insertion sites were identified based on Mb3601 reference to determine orthologous genomic sites of the IS using eggnag 5.0.

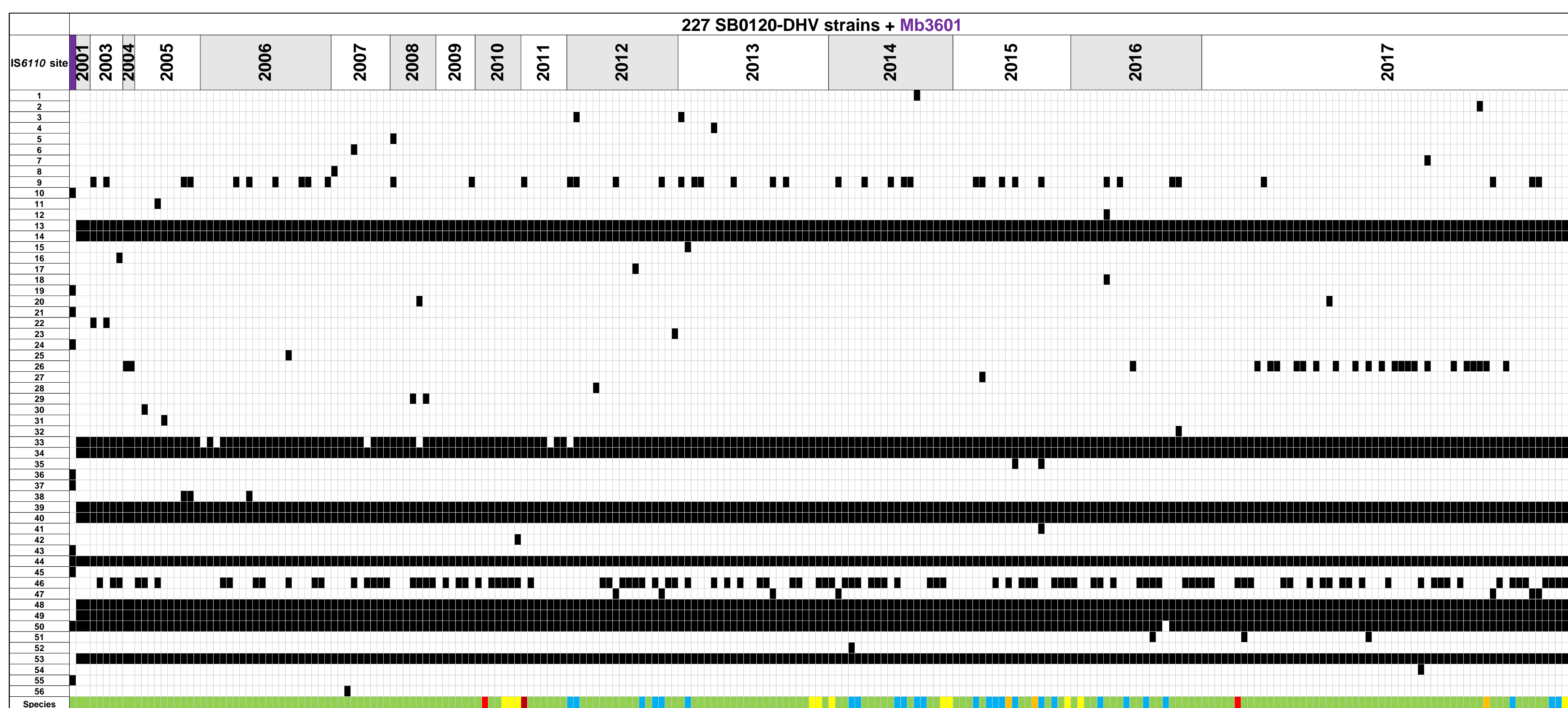
Locus	Gene in 5' of insertion site	Gene interrupted by IS6110	Gene in 3' of insertion site	Product interrupted by IS6110	Number of strains with this insertion	Group with this insertion
1-Mb3601_IS_locus	MBS3601_RS02_045	MBS3601_RS02_050	MBS3601_RS02_055	hyaluronidase/chondrosulfatase	10	Cluster A/F4 family
19-Mb3601_IS_locus*	MBS3601_RS09_095	MBS3601_RS09_100	MBS3601_RS09_105	Phospholipase D	7	Cluster I/EU3, C/SB0134 and F/EU2
33-Mb3601_IS_locus*	MBS3601_RS10_480	MBS3601_RS10_485	MBS3601_RS10_490	DNA repair helicase	12	Cluster I/EU3 and A/F4 family
43-Mb3601_IS_locus	MBS3601_RS14_500	MBS3601_RS14_505	MBS3601_RS14_510	IS6110 present in Mb3601	79	All group
50-Mb3601_IS_locus	MBS3601_RS17_200	MBS3601_RS17_205	MBS3601_RS17_210	IS6110 present in Mb3601	7	Cluster I/EU3, A/ F4 family and F/EU2

\* Several different insertion site in a same locus

Unknown function
Replication, recombination and repair
Cell wall/membrane/envelope biogenesis
Intracellular trafficking, secretion, and vesicular transport
Pseudogene



**Fig 1** Heatmap showing the presence and genomic position of IS6110 copies in genomes of French strains. The evolutionary history was inferred by using the Maximum Likelihood method (Hasegawa-Kishino-Yano model) based on 8981 wgSNP of 81 genomes (80 genomes of *Mycobacterium bovis* representing French diversity (4) and Mb3601 reference strains (2)). The strains are grouped in 7 clusters which have been previously defined (4). The unrooted tree is drawn to scale, with branch lengths measured in the number of substitutions per site.



**Fig 3** Heatmap showing the presence or absence of IS6110 in sympatric SB0120-DHV strains. *M. bovis* strains are presented by year of isolation. Illumina reads are mapped in Mb3601 reference genome which is shown in purple. Black squares show the presence of IS6110 in a specific site. The last line of the figure present the different host species of these SB0120-DHV strains (cattle, badger, wildboar, deer, roe deer and fox).

### CONCLUSIONS

The current epidemiologically **most successful *M. bovis* strains in France** have **high number of IS6110**. This striking correlation make us wonder if it could be the consequence of phenotypic modifications favouring dissemination-infection due to the genetic changes provoked by IS6110 transposition. More-in-depth analysis of the complete genome of these strains and the consequences of genetic modifications introduced by IS6110 transposition would be required to evaluate this hypothesis.

Further analyses on SB0120-DHV strains shows that the copy number and the localization of IS6110 is **very stable in time and between host species**, suggesting that host adaptation through IS6110-linked changes does not seem to have taken place (1) at least during the strain collection period.

### SYMPATRIC SB0120-DHV STRAINS

- SB0120-DHV strains have a **strong stability of the IS6110 copy number**, with an **average of 12 copies**. Among the **56 insertion sites** on Panel 2, 11 were identified in almost all SB0120-DHV strains (Figure 2). Only two sites are common between SB0120-DHV and Mb3601 (SB0120-CO).
- These strains have a **high recurrence** of their genomic position **over the time and independently of the animal species** (Figure 2).
- This stability was also observed in two other panels of SB0120-CO and F4 family multi-copy strains (data not shown).

### REFERENCES

- (1) Gonzalo Ascensio J et al PLoS Genetics, 2018
- (2) Branger M et al Genetics and Evolution 2020
- (3) Hawkey J et al BMC Genomics 2015
- (4) Hauer et al Front Microbiol 2019
- (5) Delavenne et al Bulletin épidémiologique 2019
- (6) Delavenne et al Bulletin épidémiologique 2022