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Interaction between the parasitoid *Cotesia typhae* and its host *Sesamia nonagrioides*: insights into virulence and resistance traits

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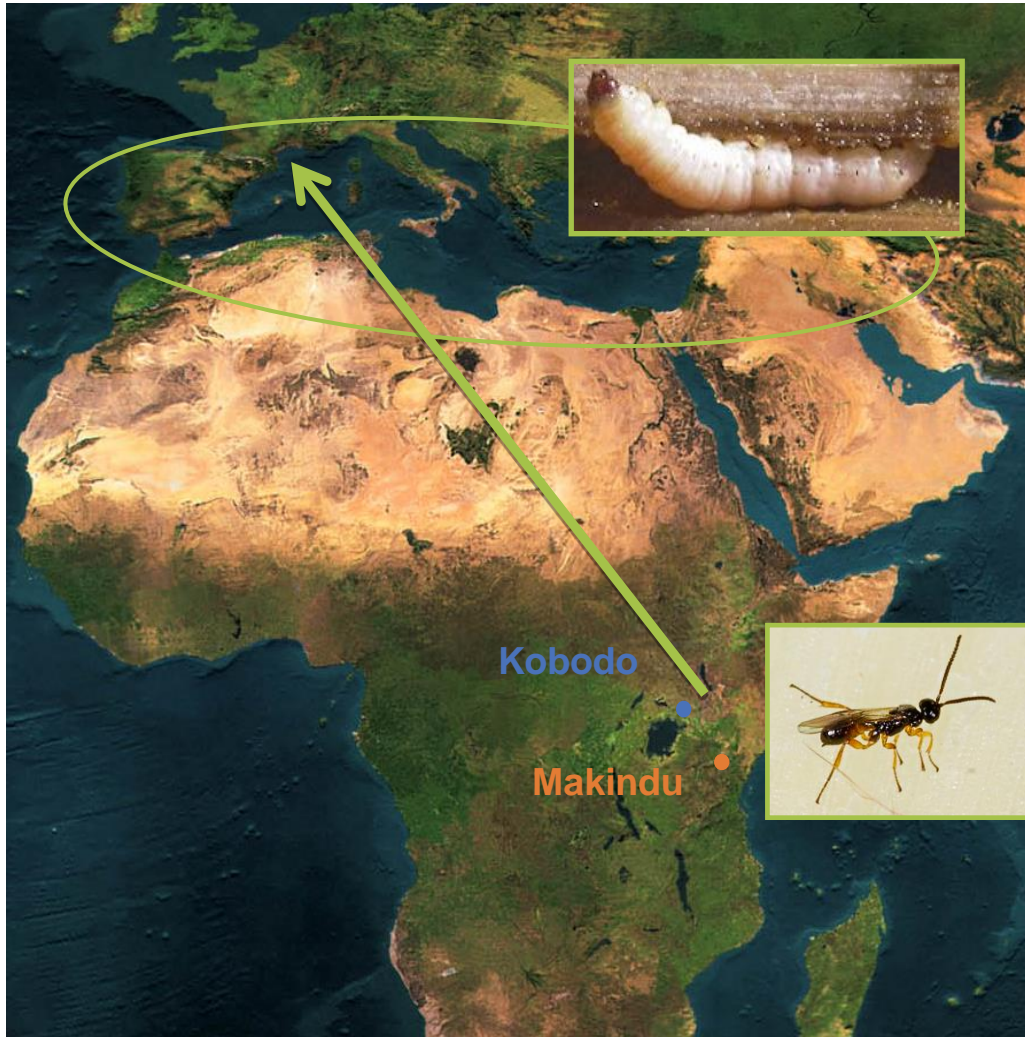


Interaction between the parasitoid *Cotesia typhae* and its host *Sesamia nonagrioides* : insights into virulence and resistance traits

Samuel GORNARD

PhD works, supervised by Florence Mougél-Imbert and Laure Kaiser-Arnauld
In collaboration with Claire Capdevielle-Dulac, Pascaline Venon and Florian Lasfont

Cotesia typhae



Cotesia typhae parasitizing its host, the larva of *Sesamia nonagrioides* (Lepidoptera : Noctuidae)

- *C. typhae* (Hymenoptera : Braconidae, Kaiser et al., 2017b) originates from Kenya
- Parasite of stem borers, specialized on *S. nonagrioides* → differentiation from other species of the *flavipes* complex
- Two diverging inbred strains reared in the lab : **Kobodo** and **Makindu** (two Kenyan localities)

Differences in virulence and resistance











On the French host population :

- **Kobodo** strain is highly virulent (V+)
- **Makindu** strain displays an inferior success, it is then weakly virulent (V-)

→ There are intraspecific differences in parasitism success

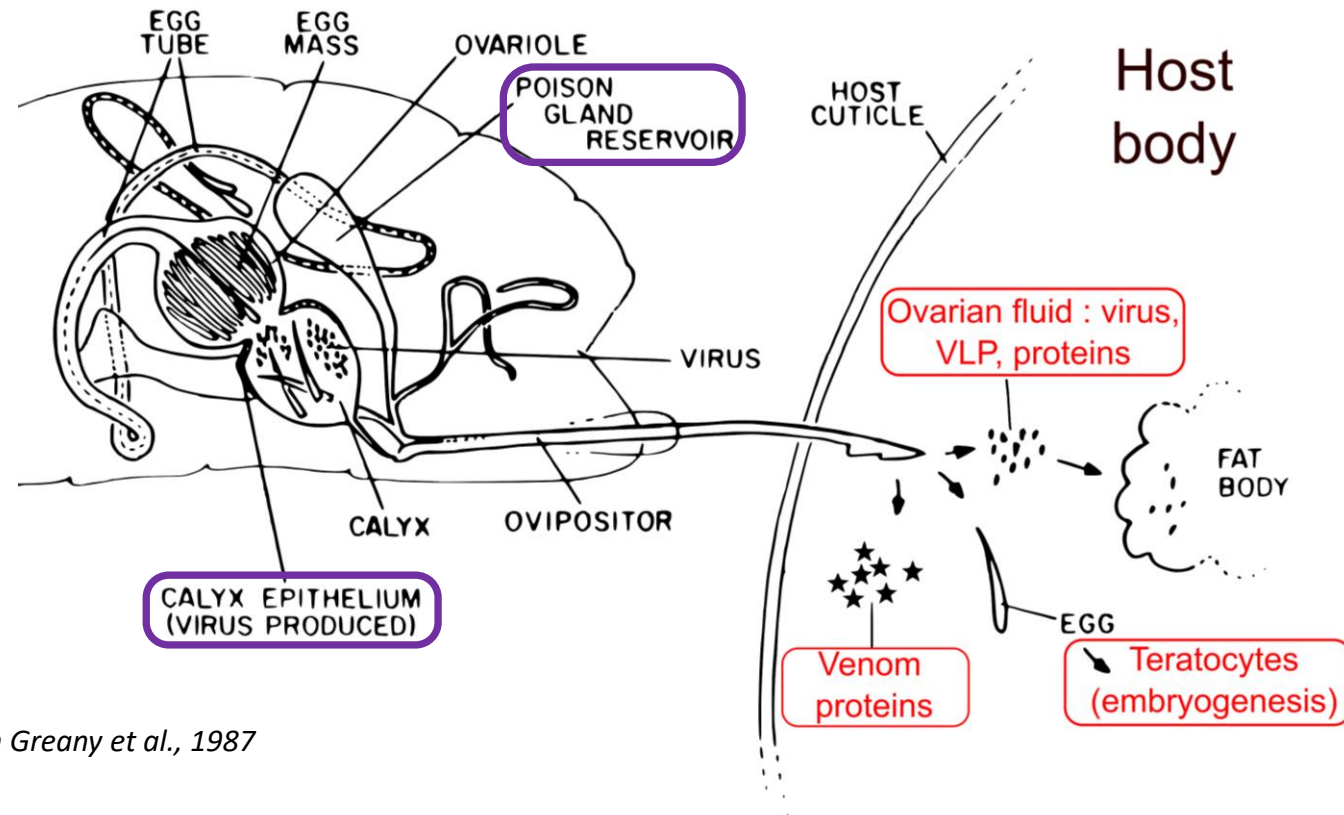
Some genomic regions (**QTL**) are involved, and especially contain venom synthesis genes

(Benoist *et al.*, 2017 ; 2020)

		Parasitoid	
		 Makindu (V-)	 Kobodo (V+)
Host	 	 39 %	 80 %
	 	 88 %	 94 %

Virulence factors of the parasitoid

Parasitoid abdomen



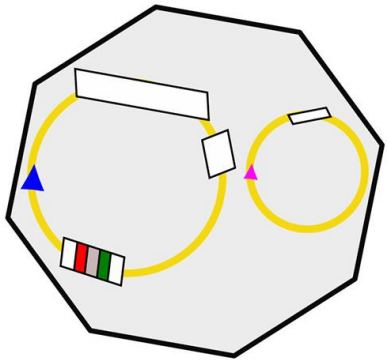
From Greany et al., 1987

Virulence factors synthesis occurs in **specialized organs** → overcome the host's immune defences

Virulence factors :

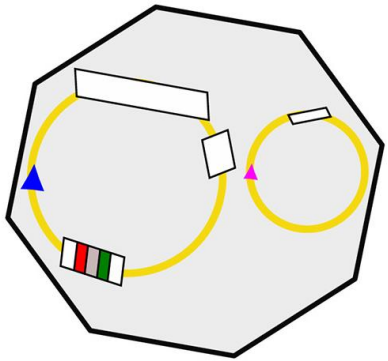
- **Venom**
- **Endosymbiotic virus** (polyDNAvirus)
- Ovarian fluid
- Virus-like particles (VLP)
- Teratocytes (embryogenesis)

Virulence genes in the parasitoid's genome

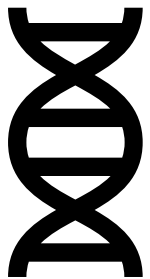


- 161 genes in proviral segments
 - + 9 out of identified segments
- (Muller et al., 2021)*

Virulence genes in the parasitoid's genome

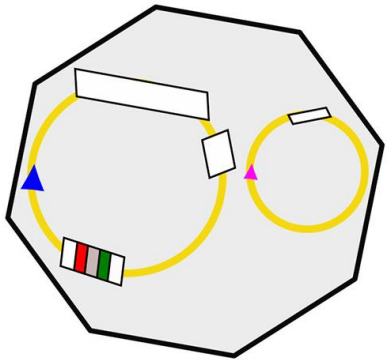


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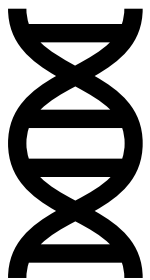


- 1448 genes in QTL
 - Involved in parasitism and reproductive success
- (Benoist et al., 2017 ; 2021)*

Virulence genes in the parasitoid's genome



- 161 genes in proviral segments
 - + 9 out of identified segments
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- 1448 genes in QTL
 - Involved in parasitism and reproductive success
- (Benoist et al., 2017 ; 2021)









Proteic blast against the iVenomDB :

- ✕ 4847 proteins
- ✕ 139 insect species
- ✓ Manually curated

Identification of 2269 potent venom proteins :

- 5 in proviral segments
- 136 in the QTL

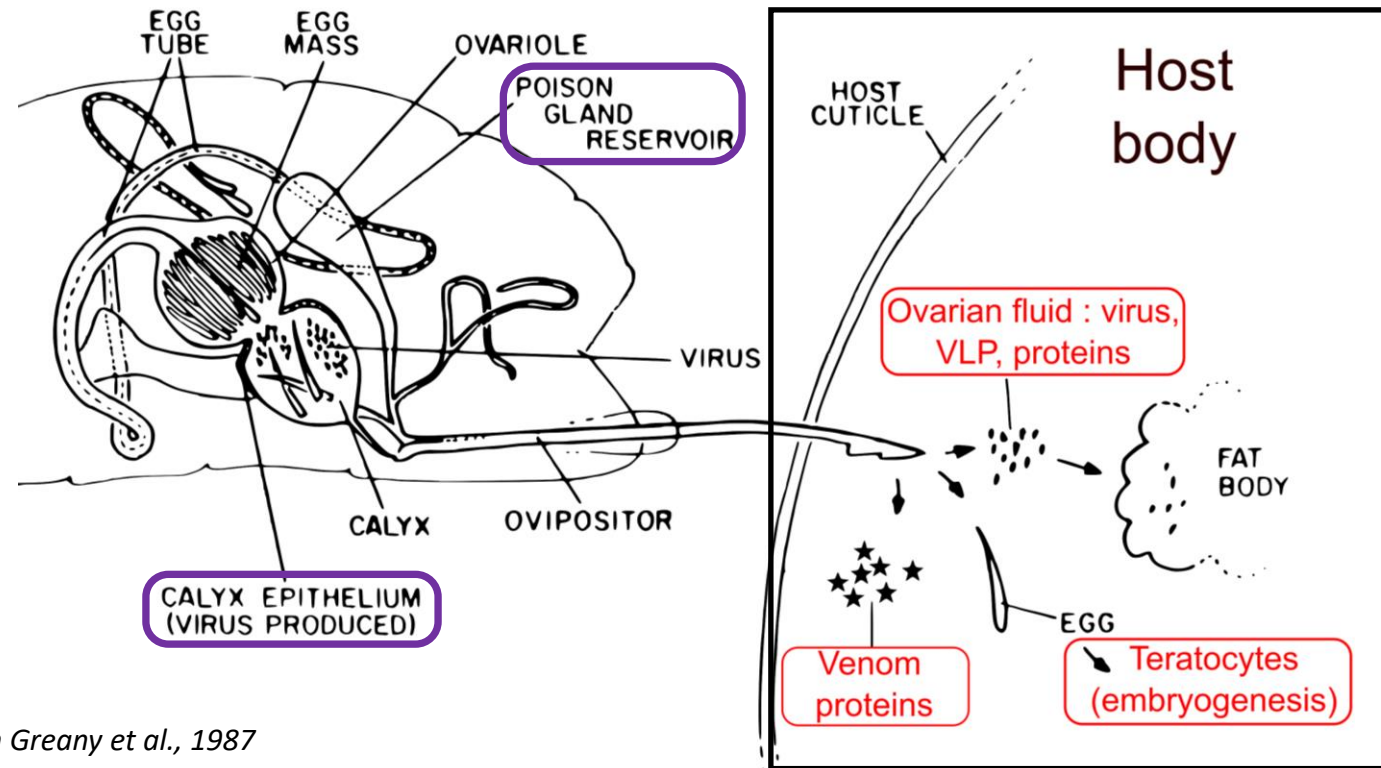
What are the molecular components involved in the difference of virulence ?

	Parasitoid	 Makindu (V-)	 Kobodo (V+)
Host			
 		+ 39 %	+++ 80 %
 		+++ 88 %	+++ 94 %

- Transcriptomic approach : differential expression
 - Parasitic stage
 - Free stages
- Proteomic approach :
 - Protein venom composition
 - Quantitative differences between strains

Comparative transcriptomic : parasitic stage

Parasitoid abdomen



From Greany et al., 1987

Parasitic stage

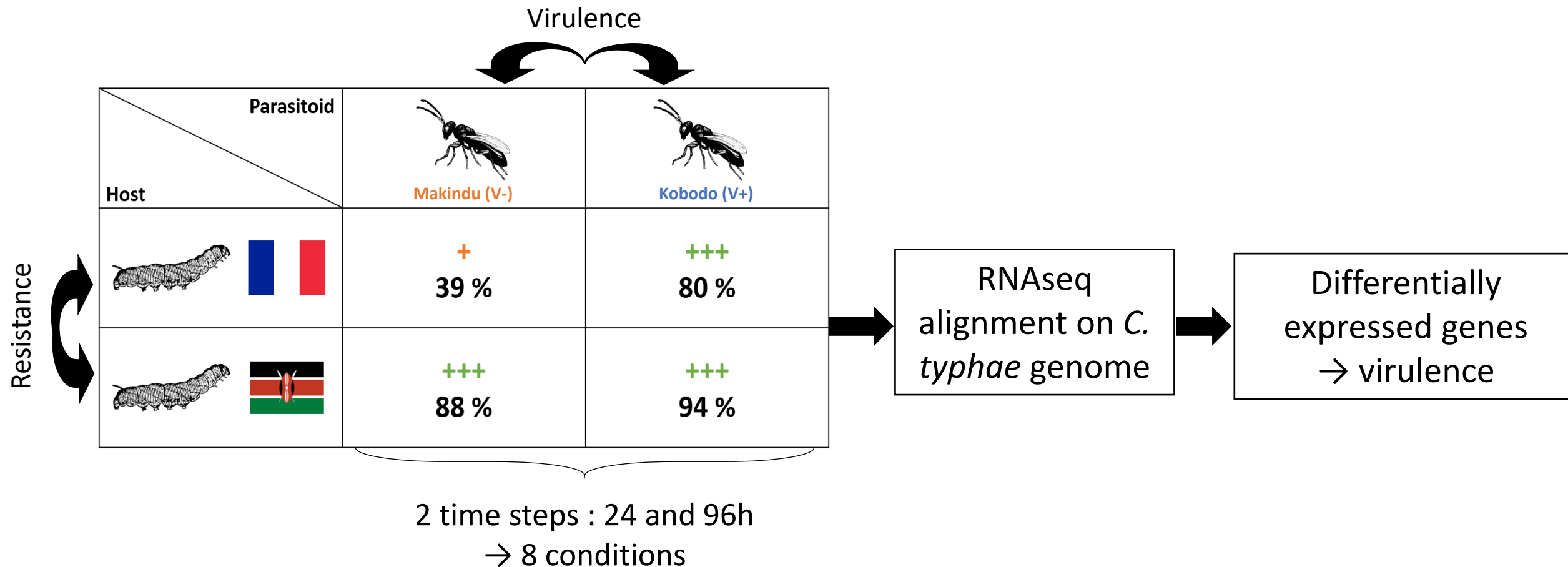
Host hemolymph
extraction

RNA - seq

Comparison across 8
conditions

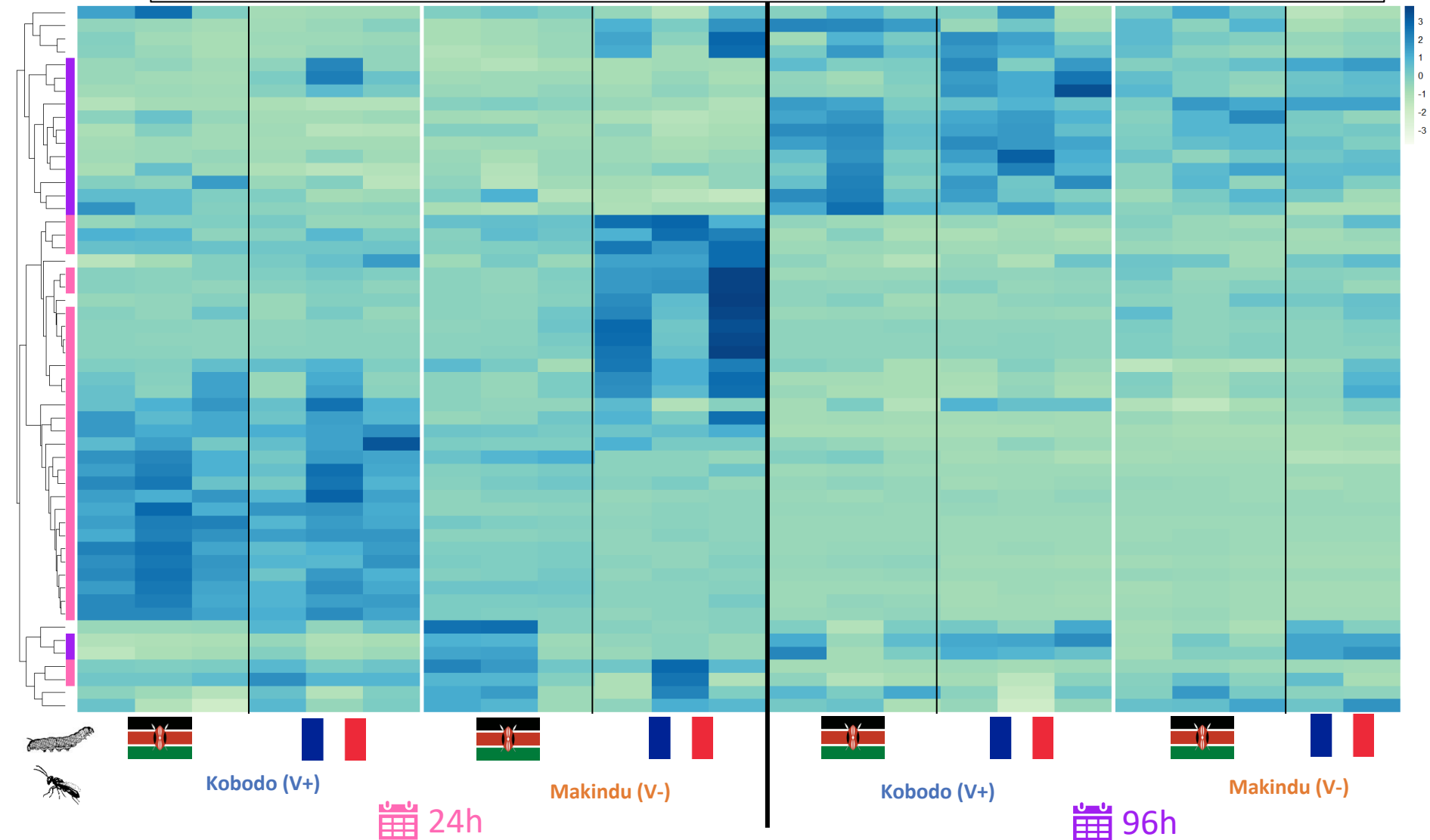
Comparative transcriptomic : parasitic stage

Host transcriptome analysis after parasitism



Results – *Cotesia* genes at 24h post-parasitism

Normalized-centered expression of the 54 genes expressed at 24h, depending on conditions

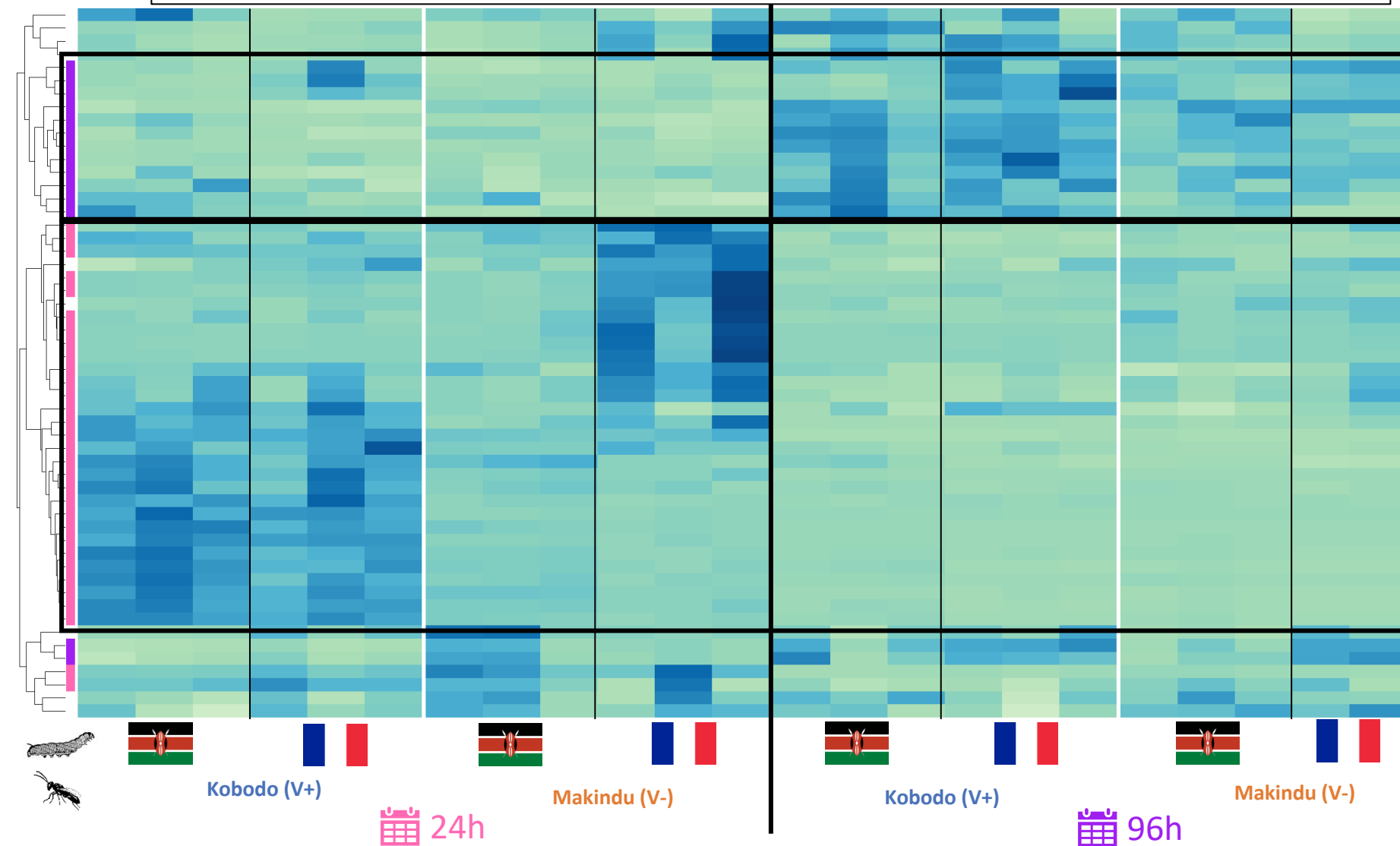


- 54 *C. typhae* genes are expressed in the host at 24h

- All of them are proviral genes

Results – *Cotesia* genes at 24h post-parasitism

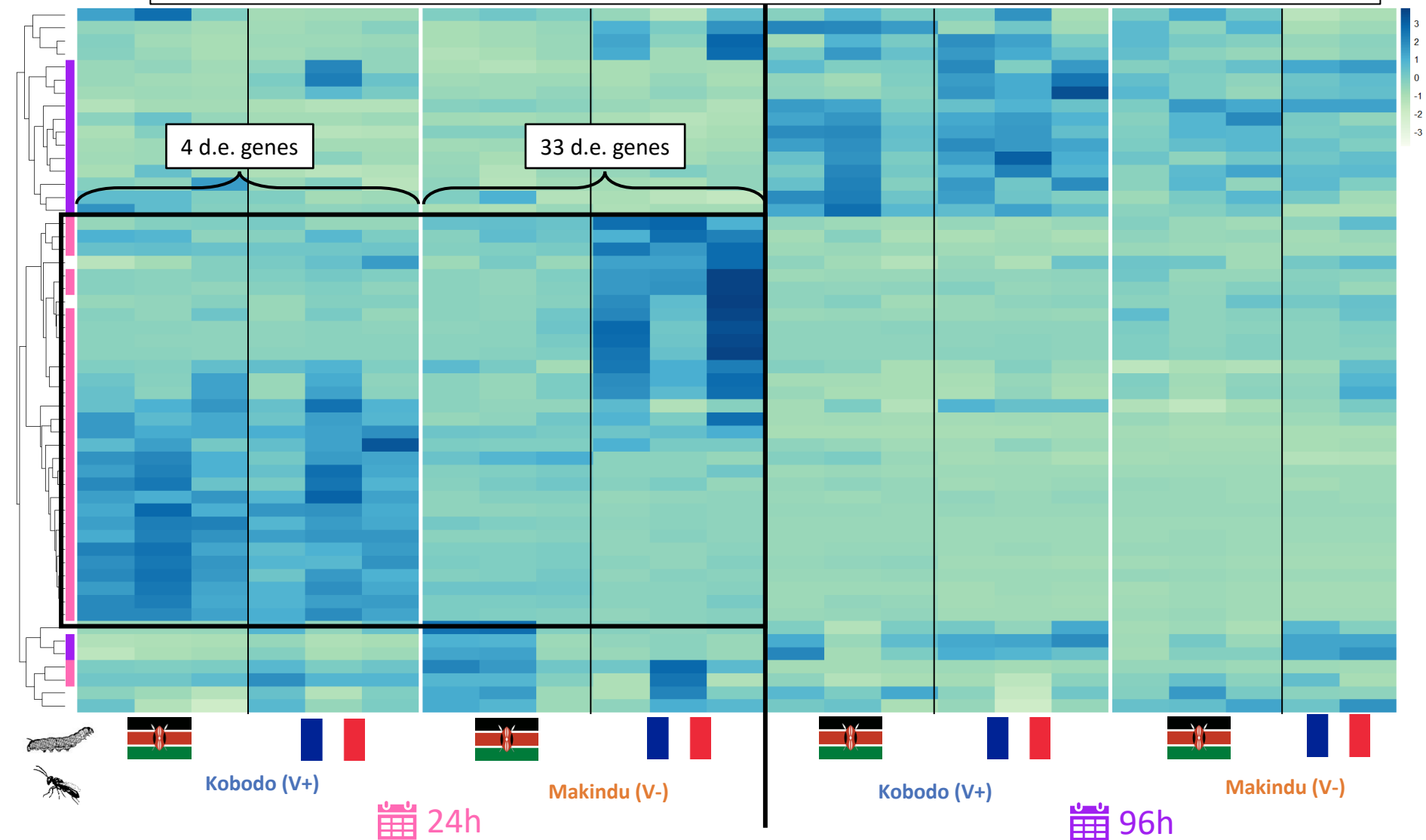
Normalized-centered expression of the 54 genes expressed at 24h, depending on conditions



- Proviral genes are mostly overexpressed at **24h** versus **96h**

Results – *Cotesia* genes at 24h post-parasitism

Normalized-centered expression of the 54 genes expressed at 24h, depending on conditions

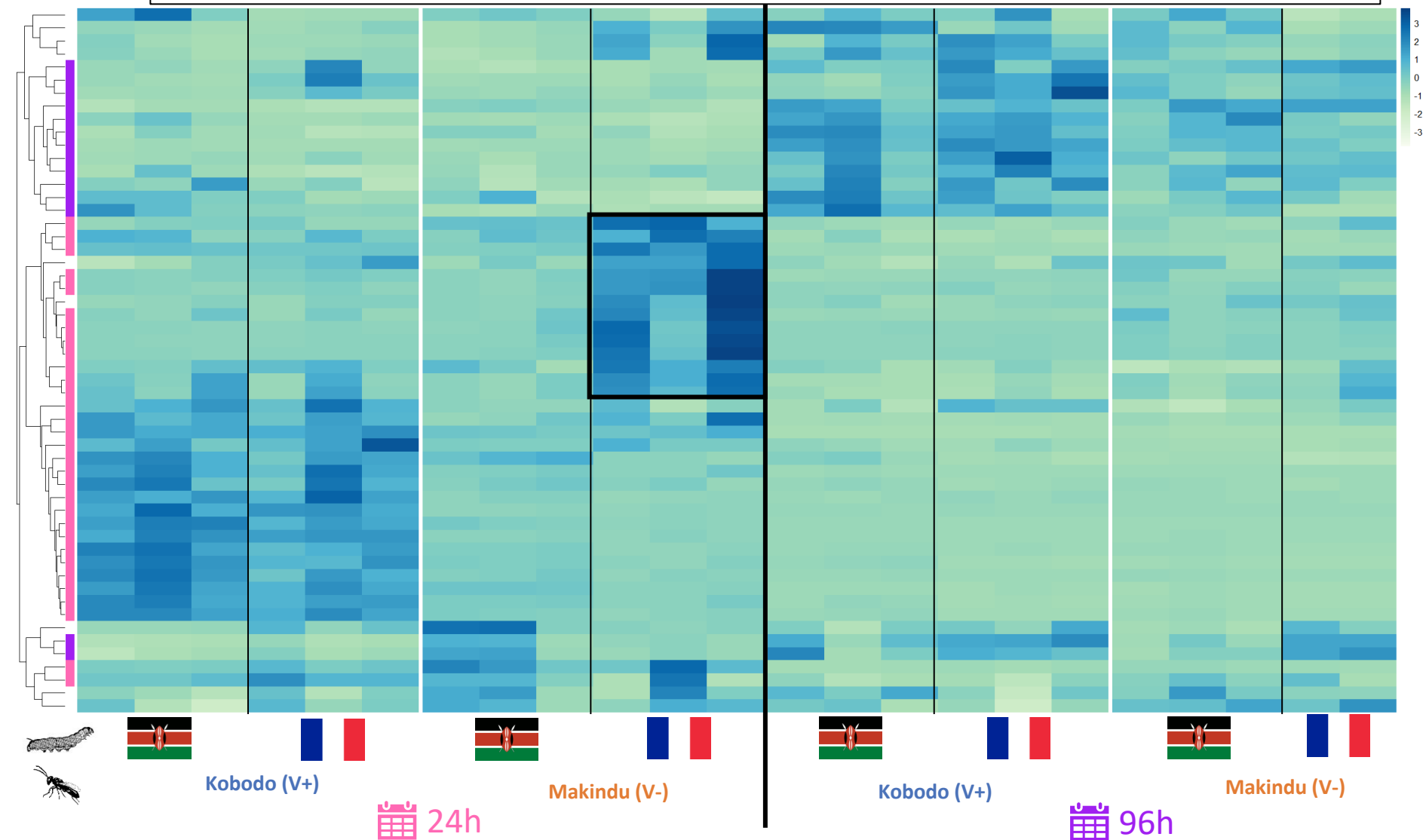


- Proviral genes are mostly overexpressed at **24h** versus **96h**

- Expression in **Kobodo (V+)** is constant. Strong overexpression of some genes in the French host for **Makindu (V-)** strain.

Results – *Cotesia* genes at 24h post-parasitism

Normalized-centered expression of the 54 genes expressed at 24h, depending on conditions



- Proviral genes are mostly overexpressed at **24h** versus **96h**

- Expression in **Kobodo (V+)** is constant. Strong overexpression of some genes in the French host for **Makindu (V-)** strain.

- 7 of those genes belong to a QTL (Benoist et al., 2021) associated with a weaker **Makindu (V-)** parasitism success

Parasitic stage expression

1. Strong expression of proviral genes at the beginning of the parasitism (24 vs 96h).
2. Differences in proviral expression between **Kobodo (V+)** and **Makindu (V-)**.
Host population has an impact → differences in virulence or resistance
3. Identification of resistance factors by analyzing host gene expression

Parasitic stage expression

1. Strong expression of proviral genes at the beginning of the parasitism (24 vs 96h).
 2. Differences in proviral expression between **Kobodo (V+)** and **Makindu (V-)**.
Host population has an impact → differences in virulence or resistance
 3. Identification of resistance factors by analyzing host gene expression
- Identification of virulence factors directly in the parasitoid

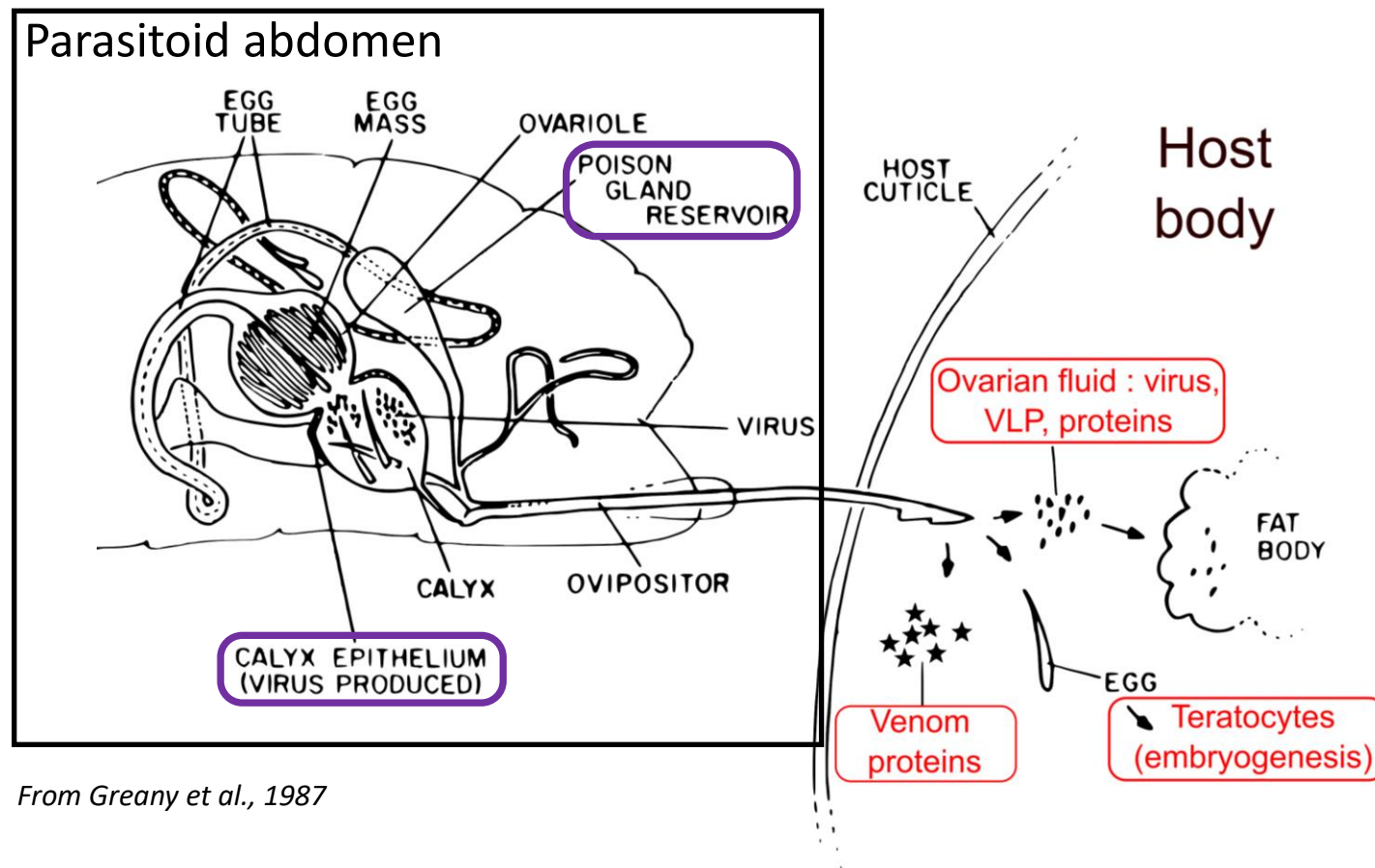
Comparative transcriptomic : free stages

Free stages

RNA extraction from parasitoid abdomen

RNA - seq

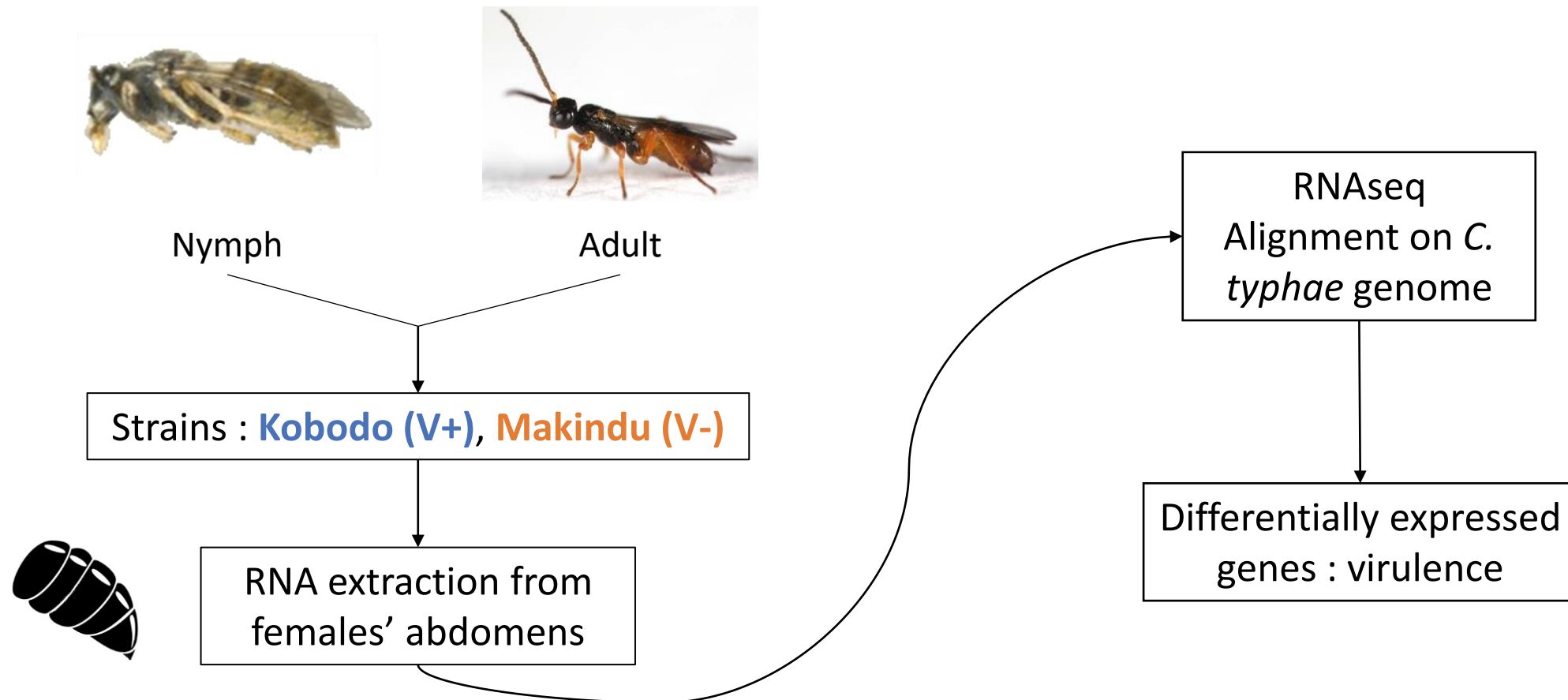
Comparison across 4 conditions



From Greany et al., 1987

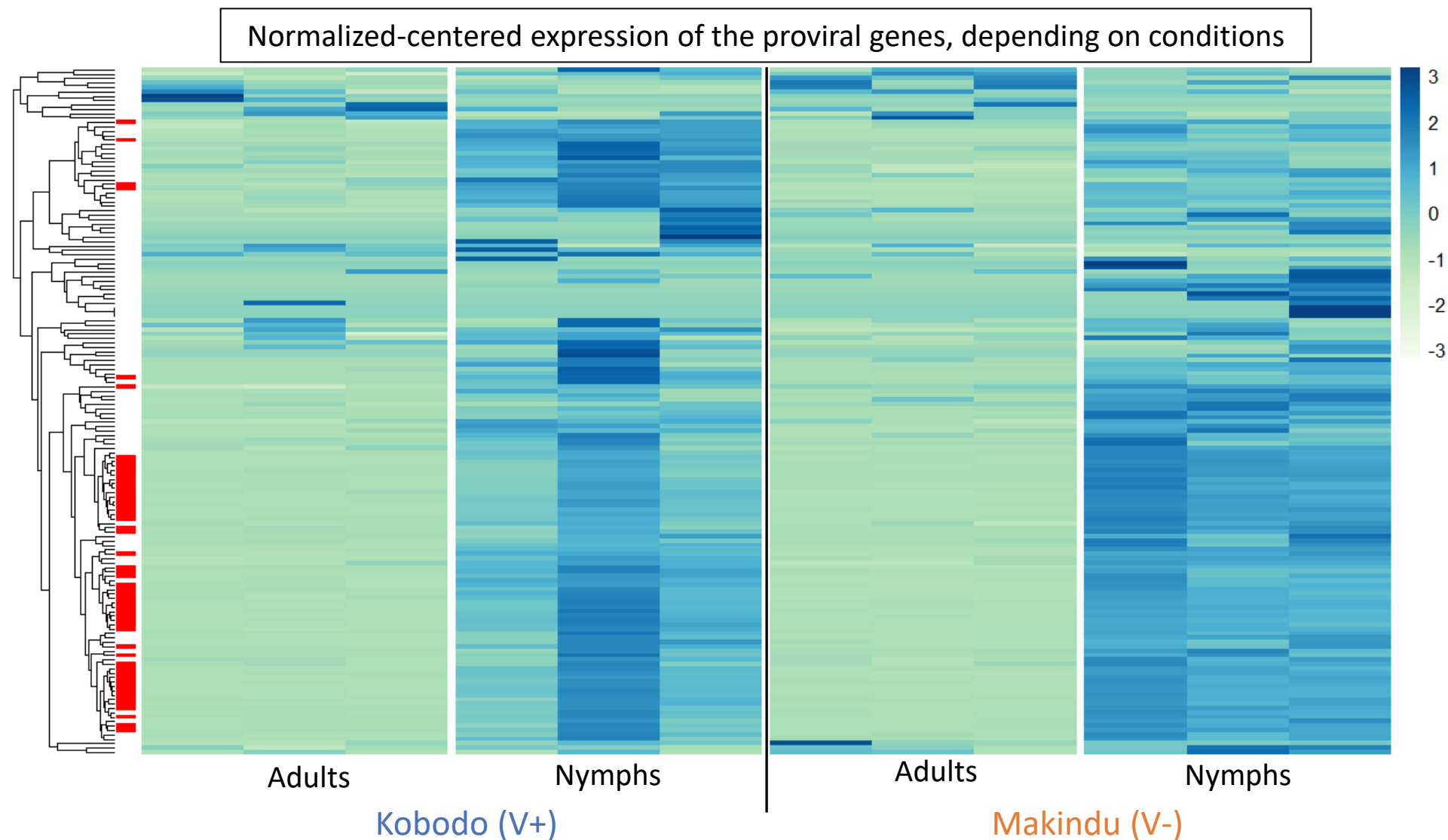
Comparative transcriptomic : free stages

Parasitoid transcriptome analysis at two life stages



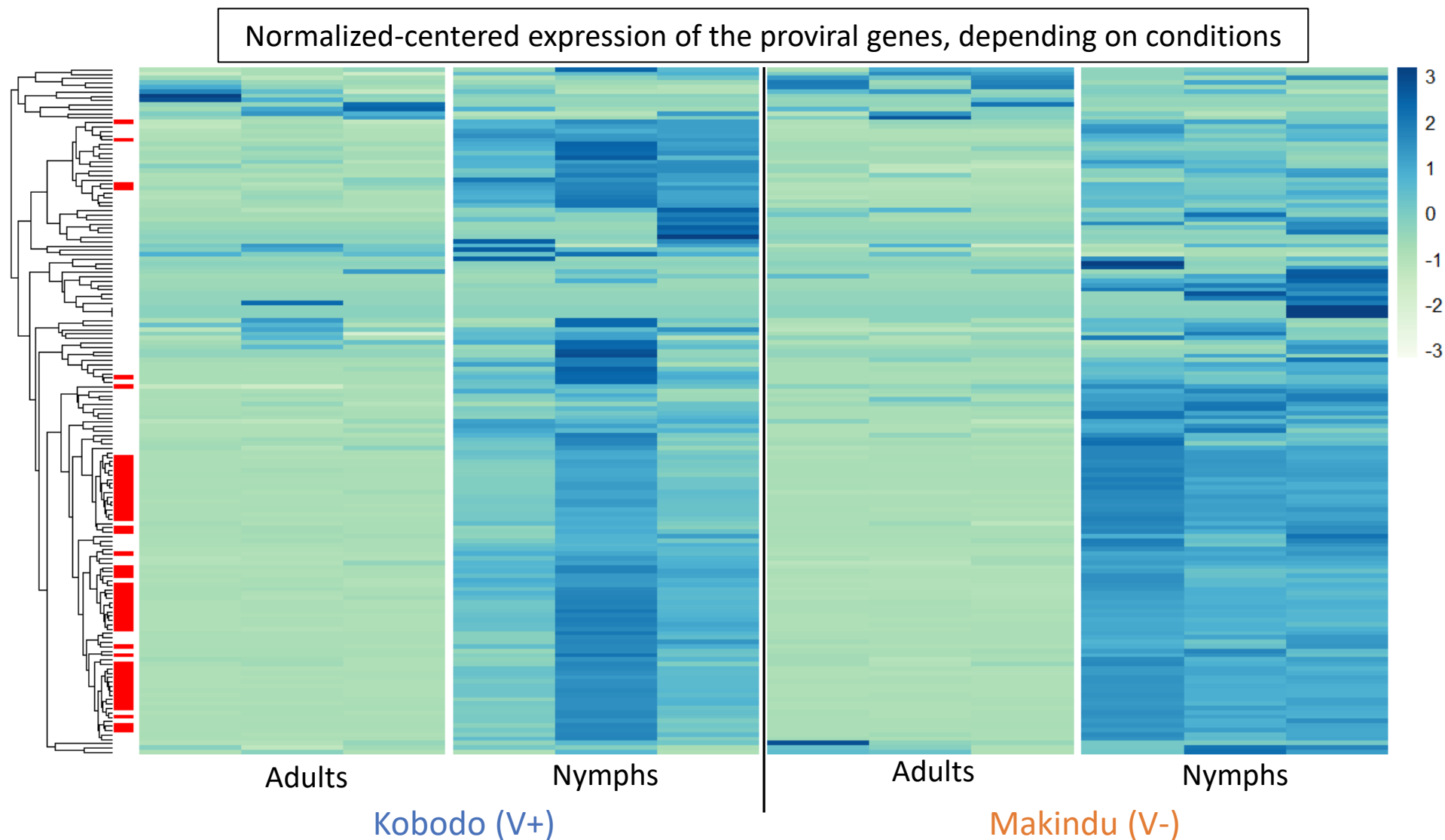
Results – Proviral genes

- On 170 proviral genes : strong expression in free stages
 - 54 **overexpressed** in the nymphs
 - 0 in the adults



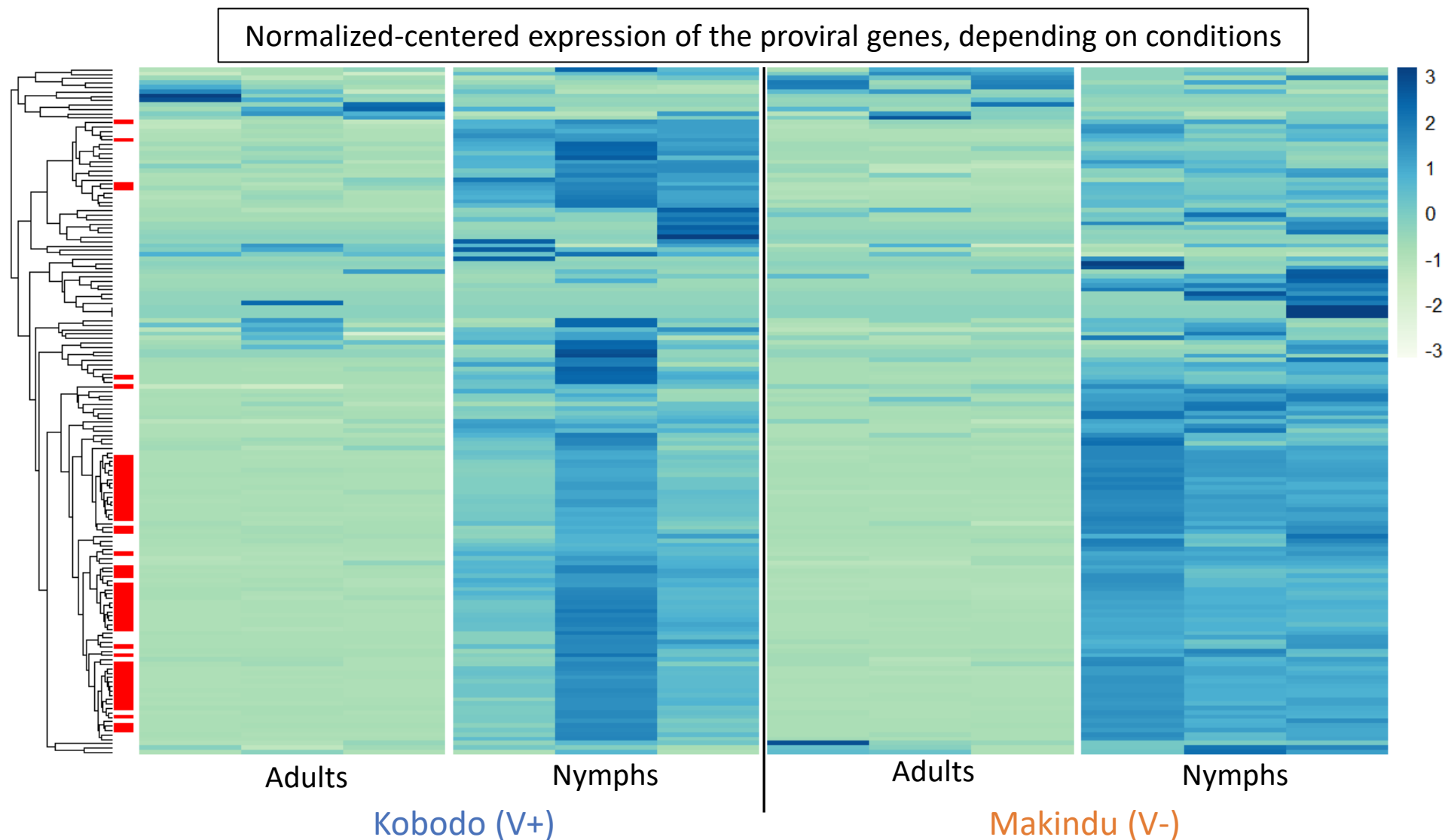
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- Only 13 d.e. proviral genes between strains (2 from the QTL)



Results – Proviral genes

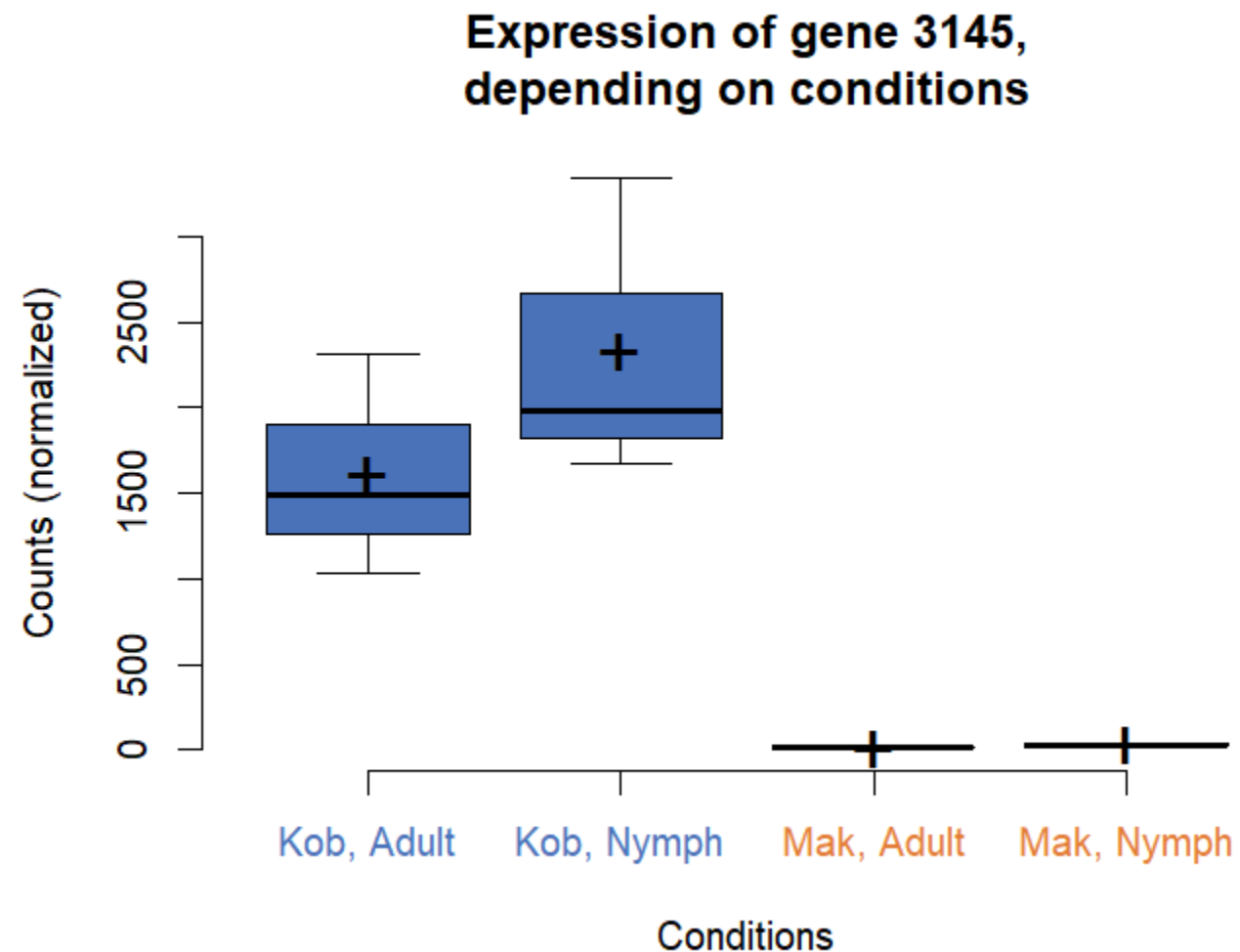
- On 170 proviral genes : strong expression in free stages
→ 54 **overexpressed** in the nymphs
→ 0 in the adults
- Only 13 d.e. proviral genes between strains (2 from the QTL)
- Mapping of the reads : detection of intron gaps
→ expression confirmed



Results – Venom genes

Focus on the potent venom genes and the genes identified by venom proteomic

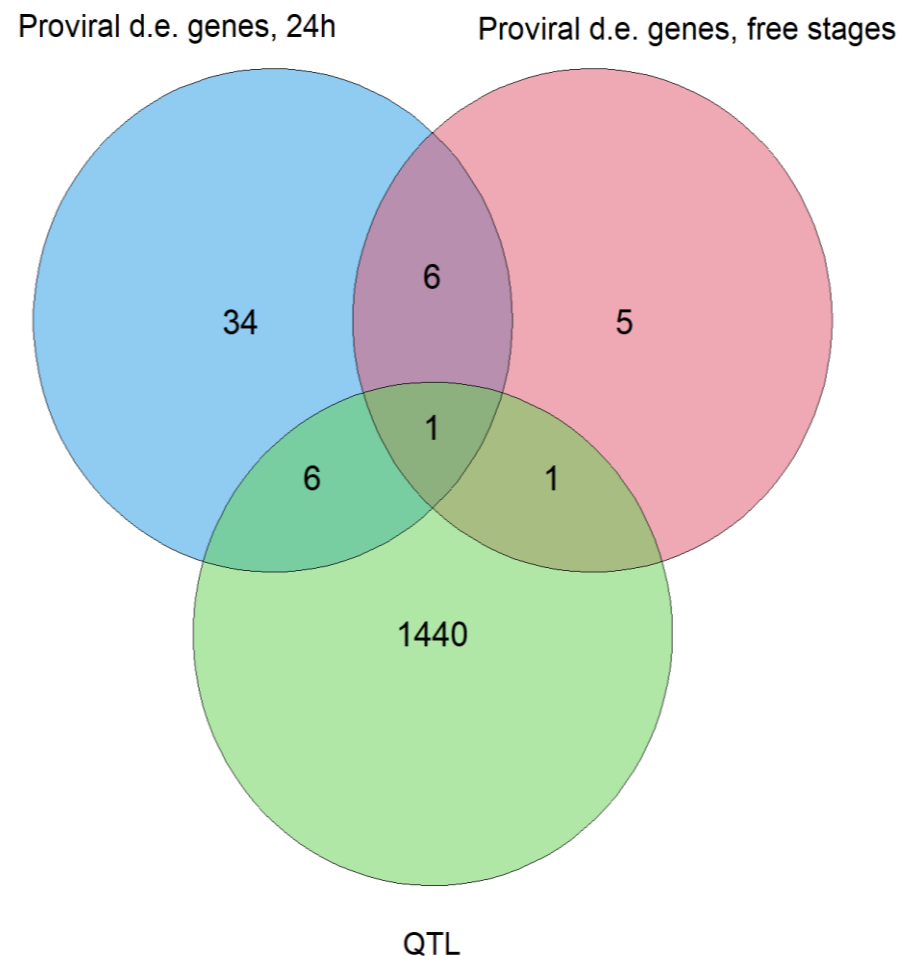
- Venom genes are mostly overexpressed in nymphs → ready to sting
- In nymphs, venom genes are mostly overexpressed in **Kobodo (V+)**
- The most underexpressed gene in **Makindu (V-)** is n° 3145
→ similar to a venom protein of *C. vestalis*, bears excretion signal peptide



Free stages expression

1. Expression of genes located in proviral segments
→ mostly in nymphs
2. Few differences in proviral genes expression between **strains**, unlike expression in the **host**
3. Venom genes : identification of candidate genes

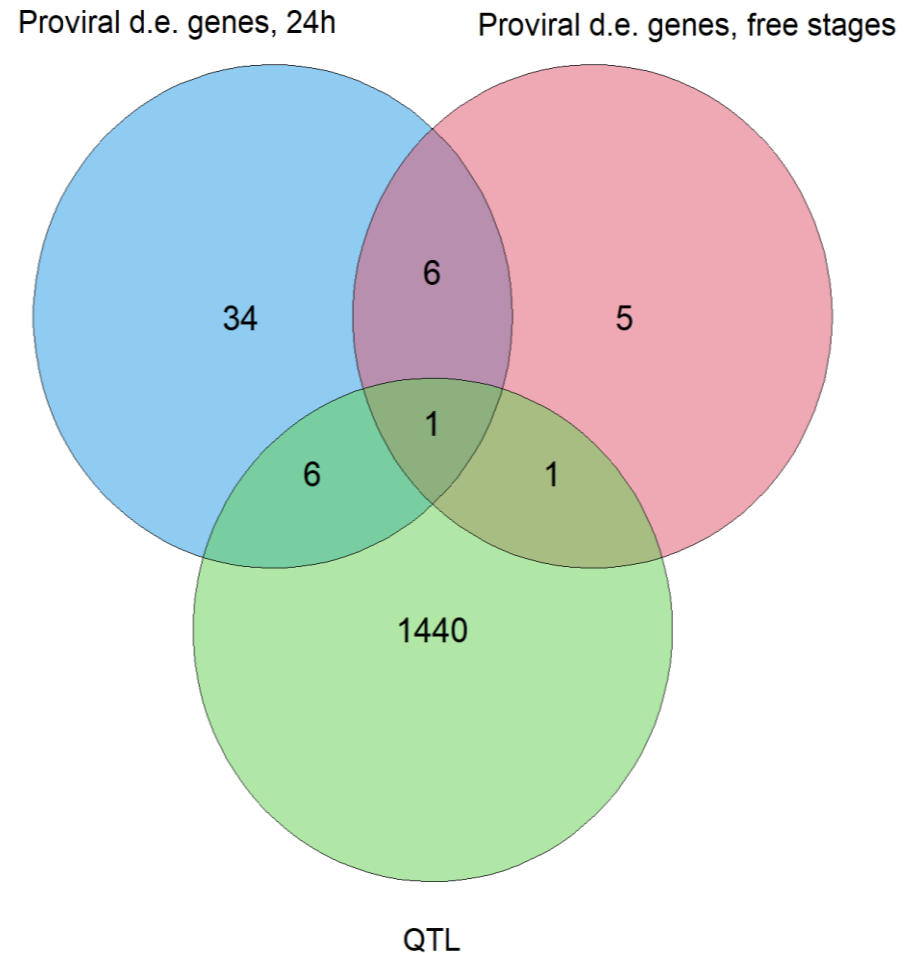
Venn diagram of common genes between Kob/Mak d.e. proviral genes and QTL



Free stages expression

1. Expression of genes located in proviral segments
→ mostly in nymphs
 2. Few differences in proviral genes expression between **strains**, unlike expression in the **host**
 3. Venom genes : identification of candidate genes
- Explore proteic composition of the venom

Venn diagram of common genes between Kob/Mak d.e. proviral genes and QTL



Proteomic – Venom

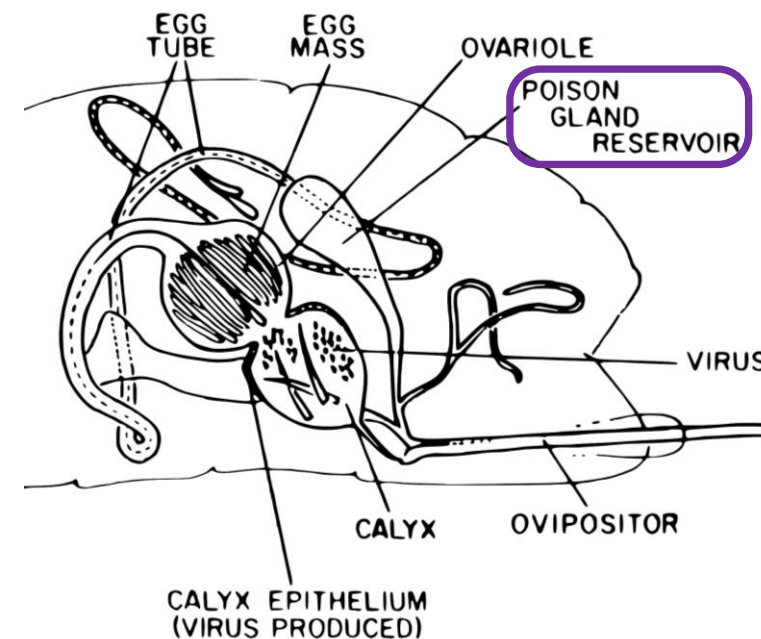
Proteomic **venom** comparison
between Kobodo and Makindu

Link with :

- QTL genes
- RNA-seq on the parasitoid

Identification of candidate genes

Parasitoid abdomen



From Greany et al., 1987

Citer pappso

Results – Venom components

Detection of **763 proteins**

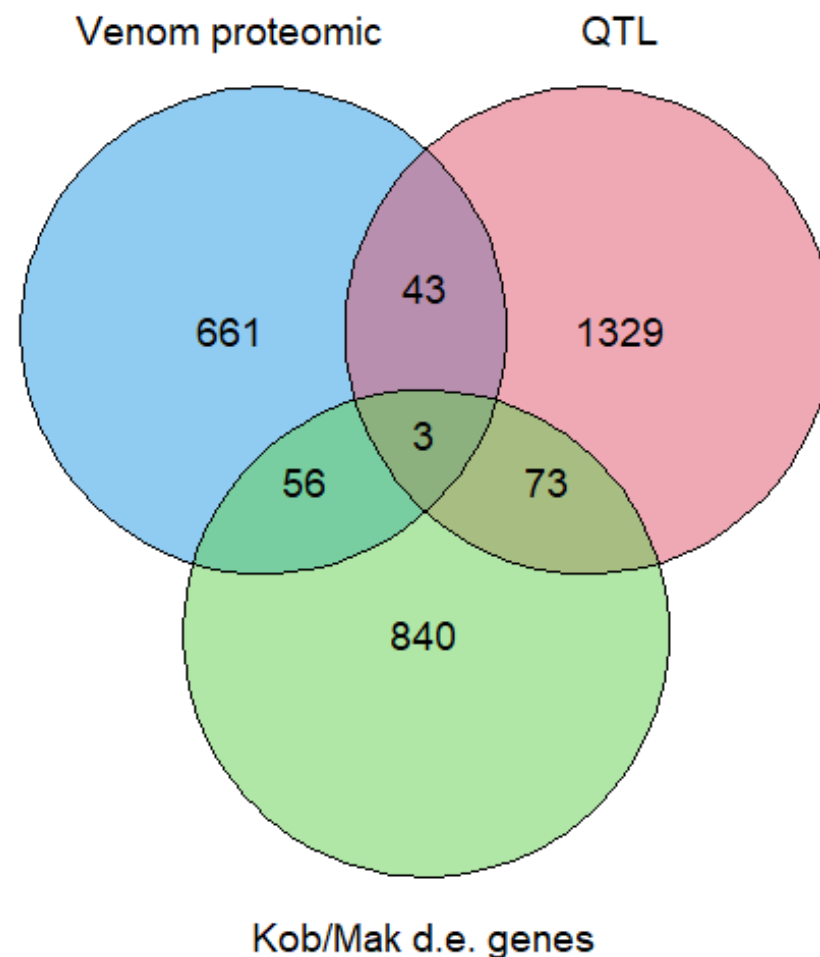
→ Refinement of the genome annotation

3 of the genes encoding those proteins are both **d.e. between strains** and inside a **QTL**.

2 are underexpressed in Makindu (V-), and bear an excretion signal :

- N° 9342, identified as a venom metalloprotease
- N° 6687, a beta-glucuronidase conserved in hymenopteran

Venn diagram of common genes between used methods

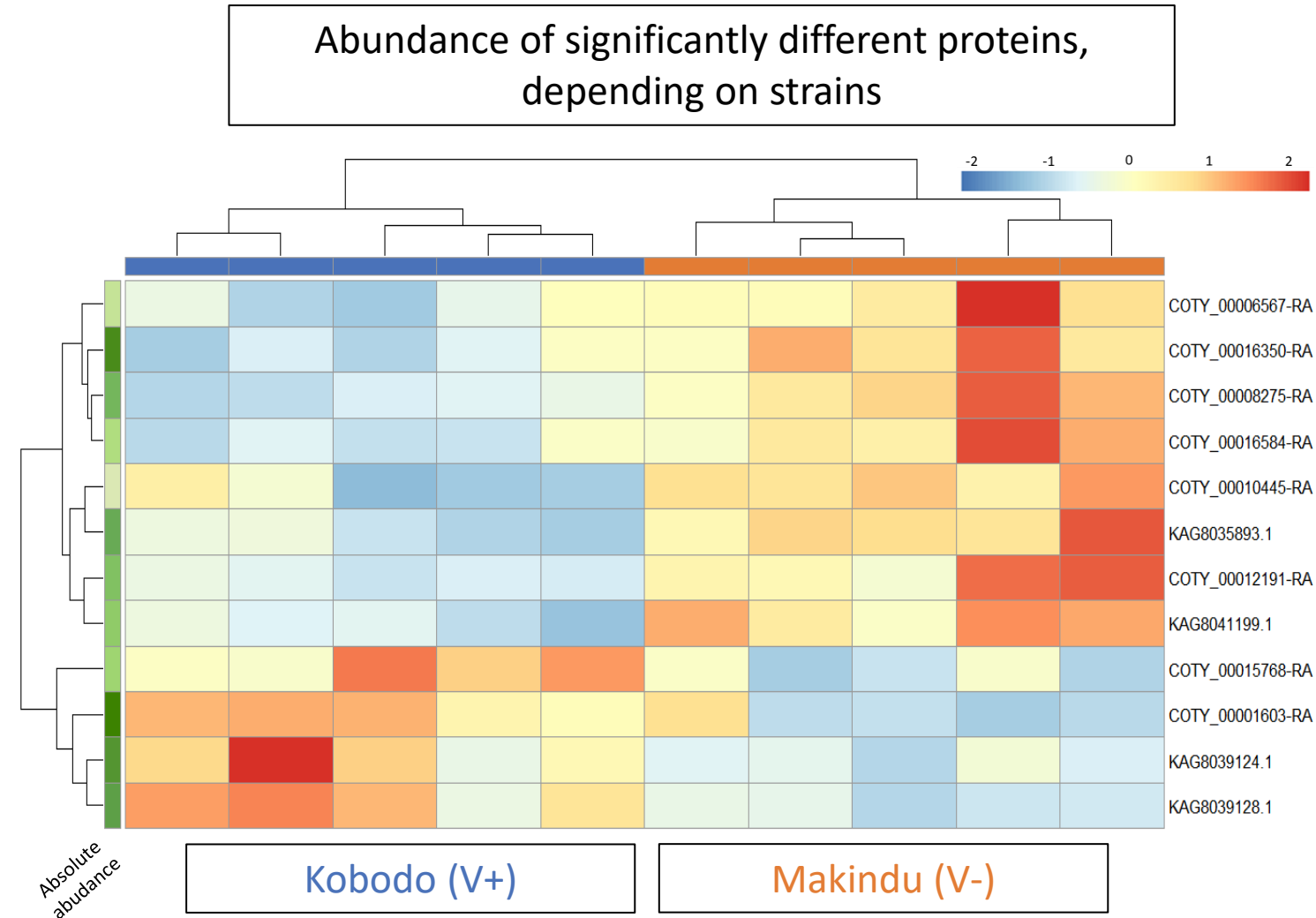


Results – Quantitative analysis

- Protein abundance comparison between strains after normalization and quality control
- Selection of 372 proteins (reduction of tissue contamination) :
 - Bearing an excretion signal peptide
 - Identified as potent venom (iVenomDB)

Results – Quantitative analysis

- Protein abundance comparison between strains after normalization and quality control
- Selection of 372 proteins (reduction of tissue contamination) :
 - Bearing an excretion signal peptide
 - Identified as potent venom (iVenomDB)
- 12 proteins are differentially abundant between strains

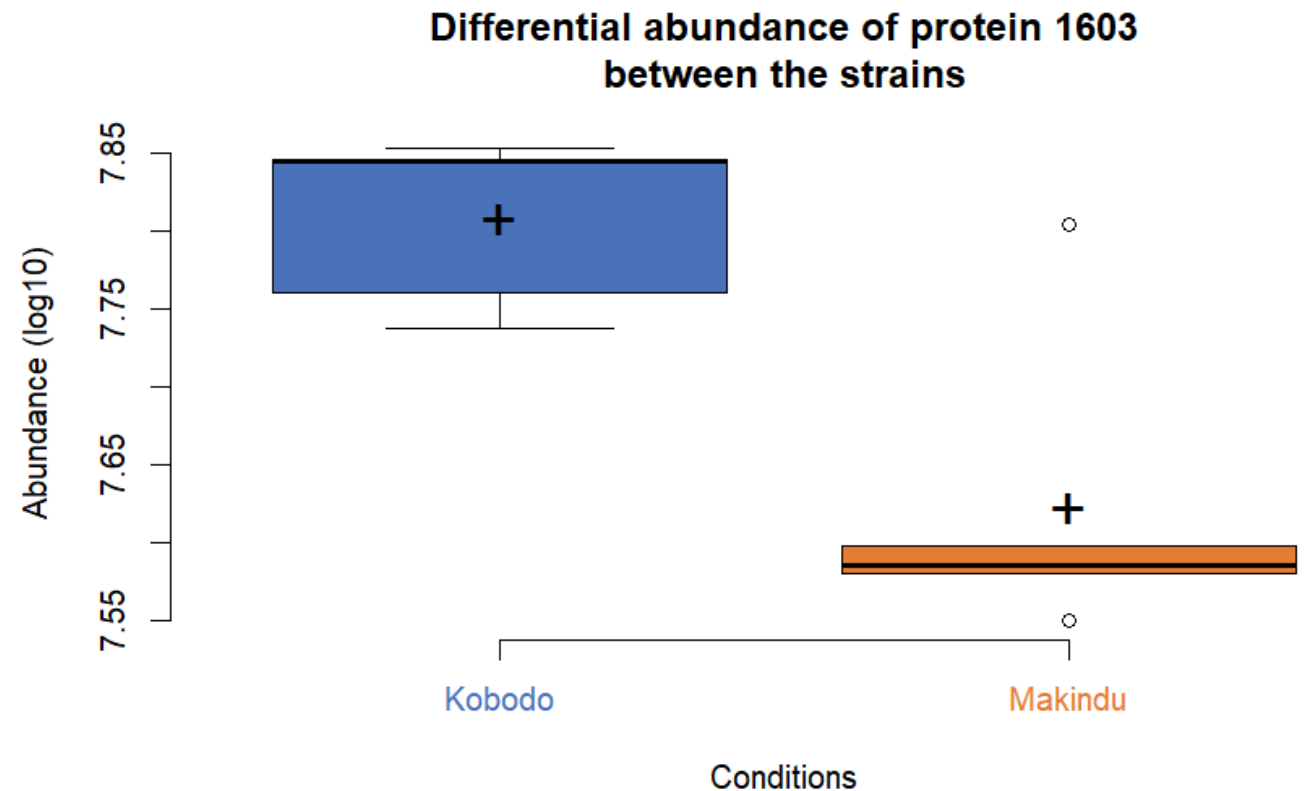


Results – Quantitative analysis

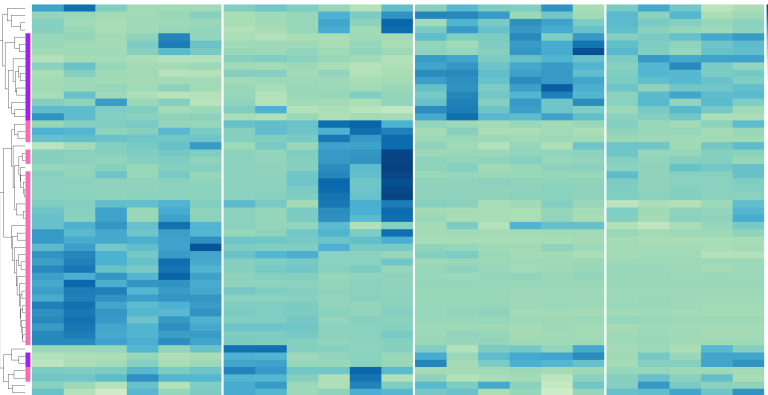
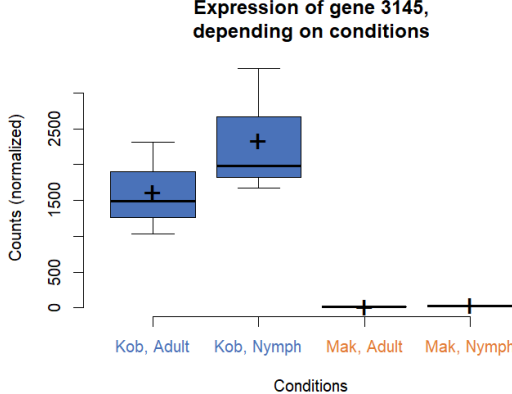
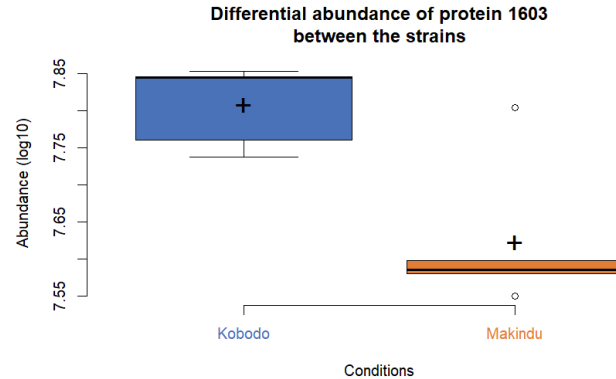
8 proteins are overabundant in **Makindu (V-)**, against 4 in **Kobodo (V+)**.

Among those 4, the protein coded by gene n° 1603 :

- Overexpressed gene in **Kobodo** (RNA-seq)
- Identified as potent venom protein (aminopeptidase)
- Is the most generally abundant protein among the 12
- Candidate gene → to be demonstrated



General conclusion

Transcriptomic Free stages	Transcriptomic Parasitic stages	Proteomic Venom
<ul style="list-style-type: none"> ● Impact of the French host strain on Makindu (V-) proviral gene expression (24h) 	<ul style="list-style-type: none"> ● Expression of proviral genes in the free stages ● Expression of venom genes 	<ul style="list-style-type: none"> ● Venom components ● Identification of a small amount of candidate genes
→ QTL investigation	→ Candidate : n° 3145	→ Candidate : n° 1603
	<p>Expression of gene 3145, depending on conditions</p> 	<p>Differential abundance of protein 1603 between the strains</p> 

Contributions

Authorship

Florence Mougel-Imbert

Claire Capdevielle-Dulac

Laure Kaiser-Arnauld

Pascaline Venon

Florian Lasfont

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Sabrina Bothorel

Taiadjana Fortuna-Marques

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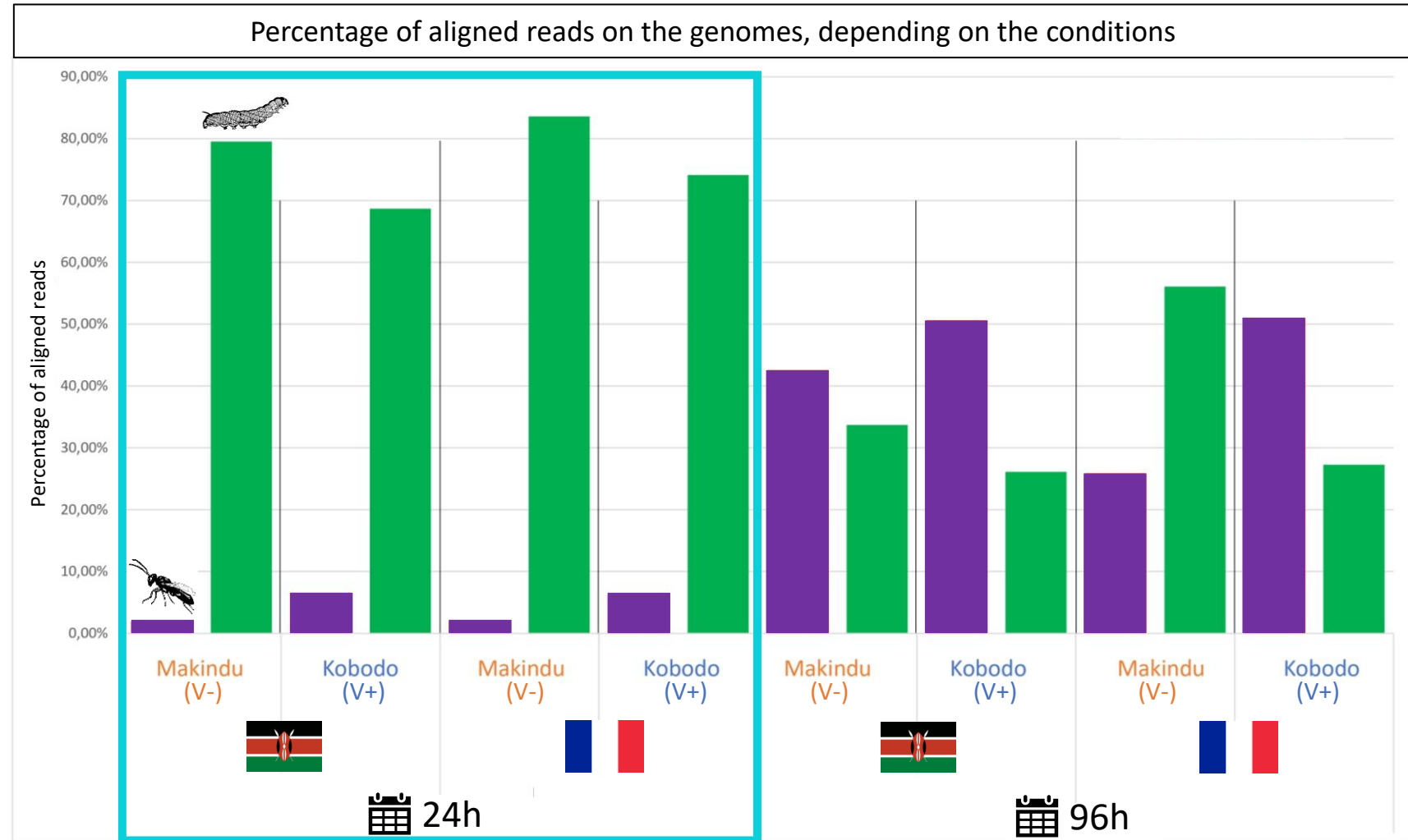




Thank you for listening !

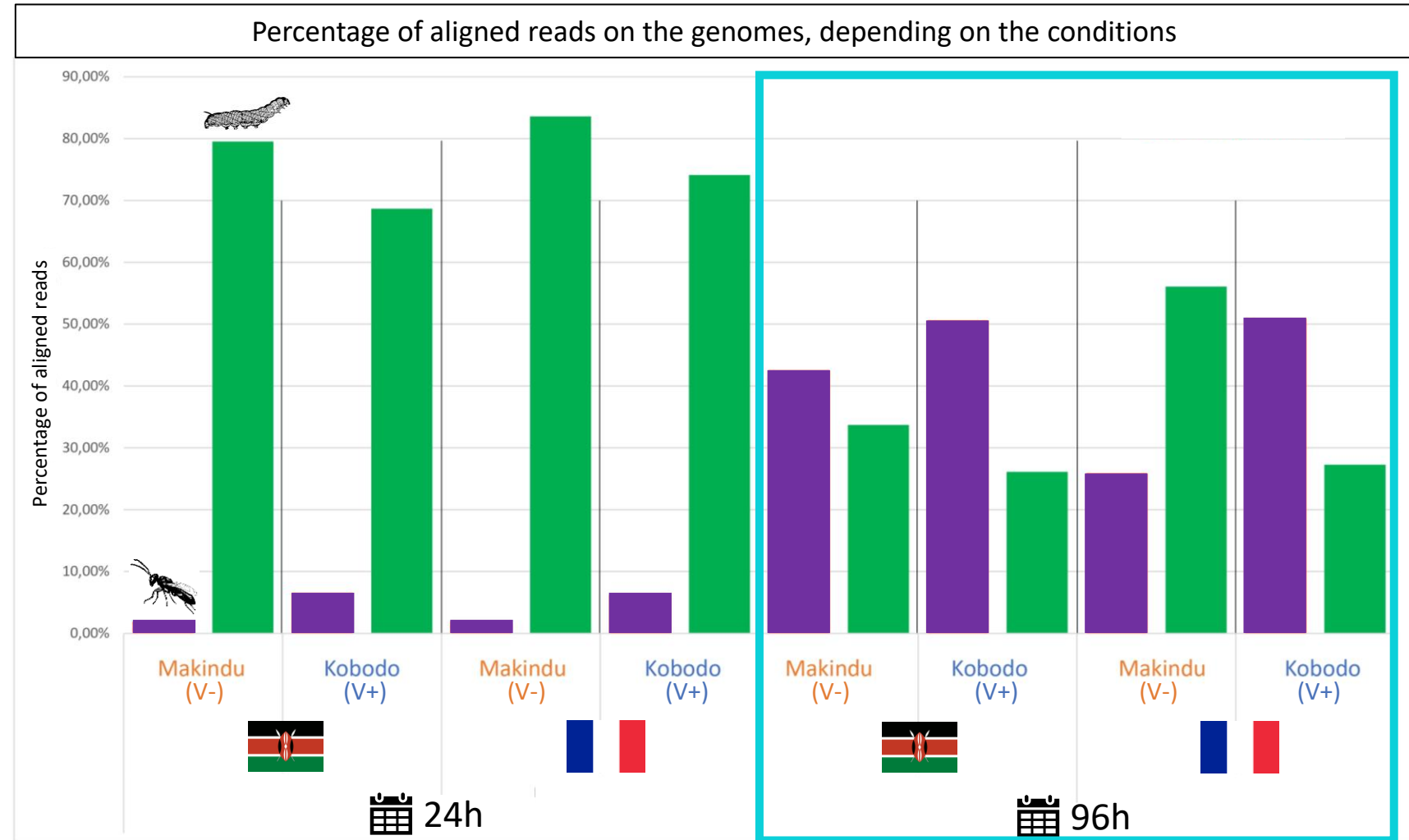
Results - Mapping

- 24 h : 54 *C. typhae* expressed, all proviral



Results - Mapping

- 24 h : 54 *C. typhae* expressed, all proviral
- 96h : 5245 *C. typhae* genes expressed



Results - Mapping

- 24 h : 54 *C. typhae* expressed, all proviral
- 96h : 5245 *C. typhae* genes expressed
- Weaker alignment percentage for **Makindu (V-)** on the French host → resistance

