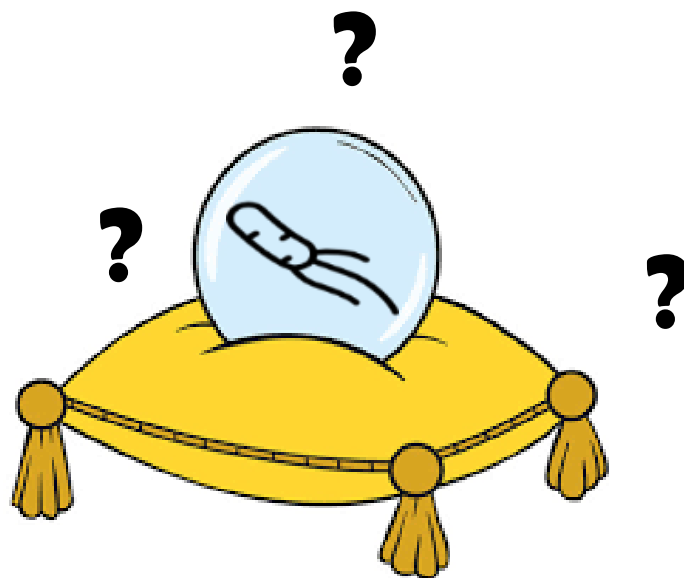


The use of Whole Genome Sequencing in Risk Assessment

The cold smoked salmon-related listeriosis risk model

Workshop: Evaluating the risk from the presence of
L. monocytogenes in RTE meat products
February 15th 2019, Athens

Lena FRITSCH,
Jean-Christophe AUGUSTIN,
Laurent GUILLIER

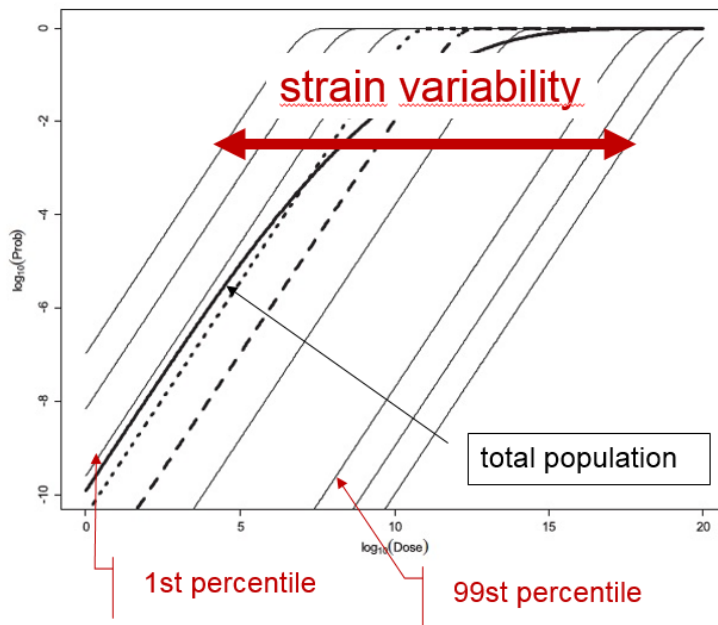


Sources of variability

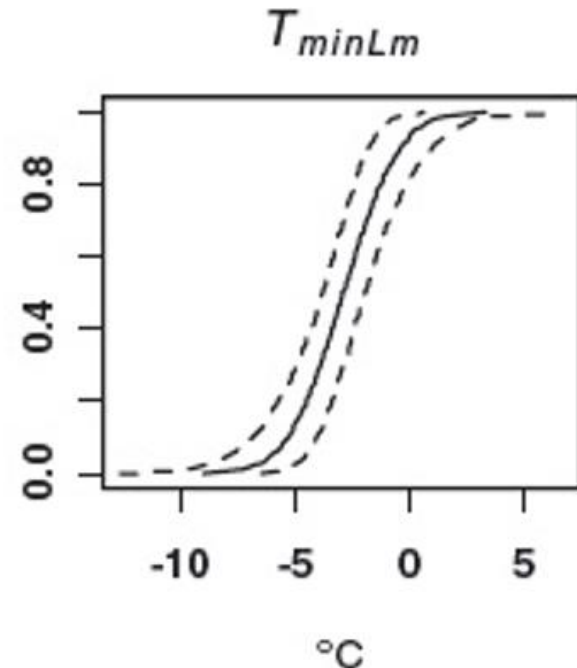
- Ex. dose-response variability

Variability across *L. monocytogenes* strains

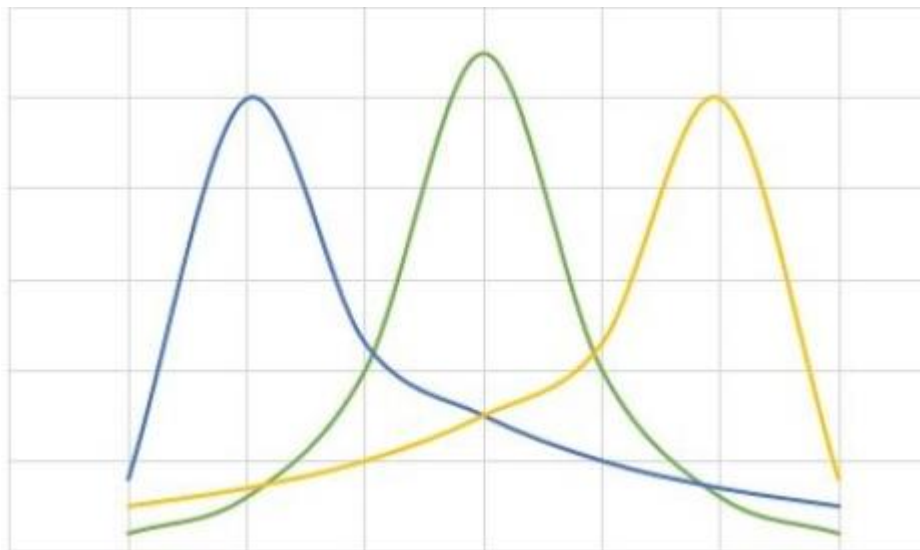
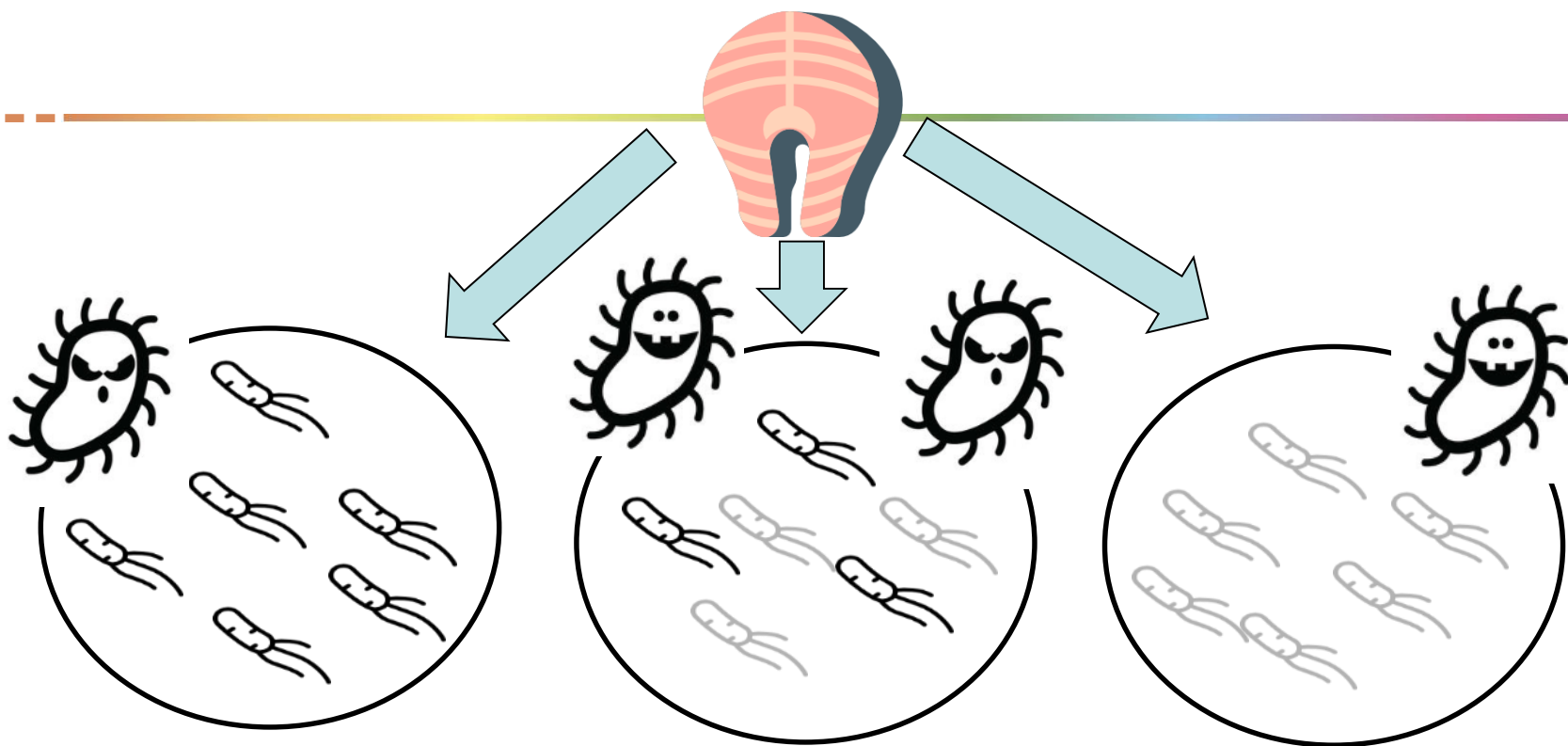
Log(*r*) increasing from more than 7 log between the 1st and 99th percentiles



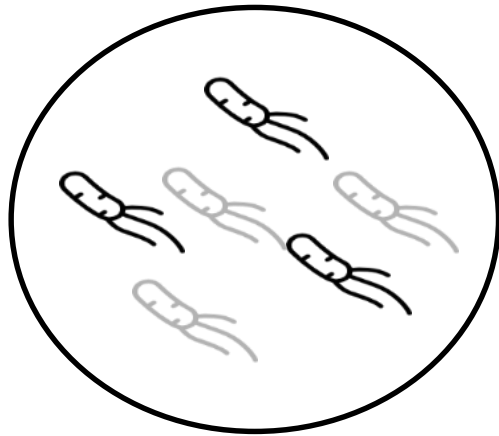
(Pouillot et al., 2015)



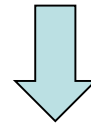
T_{min} ~ N(-2.9°C, 1.9°C)



Genome wide association studies (GWAS)



**Find associations
between genetic
variations and
observable traits**



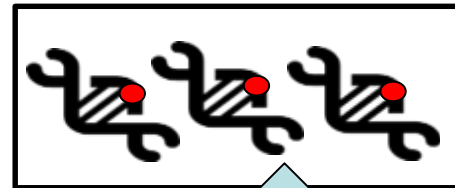
Biomarker « X »

- **Genes**
- **SNPs**
-

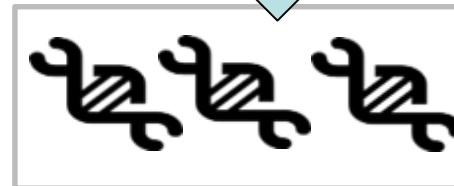
Trait

DNA sequencing

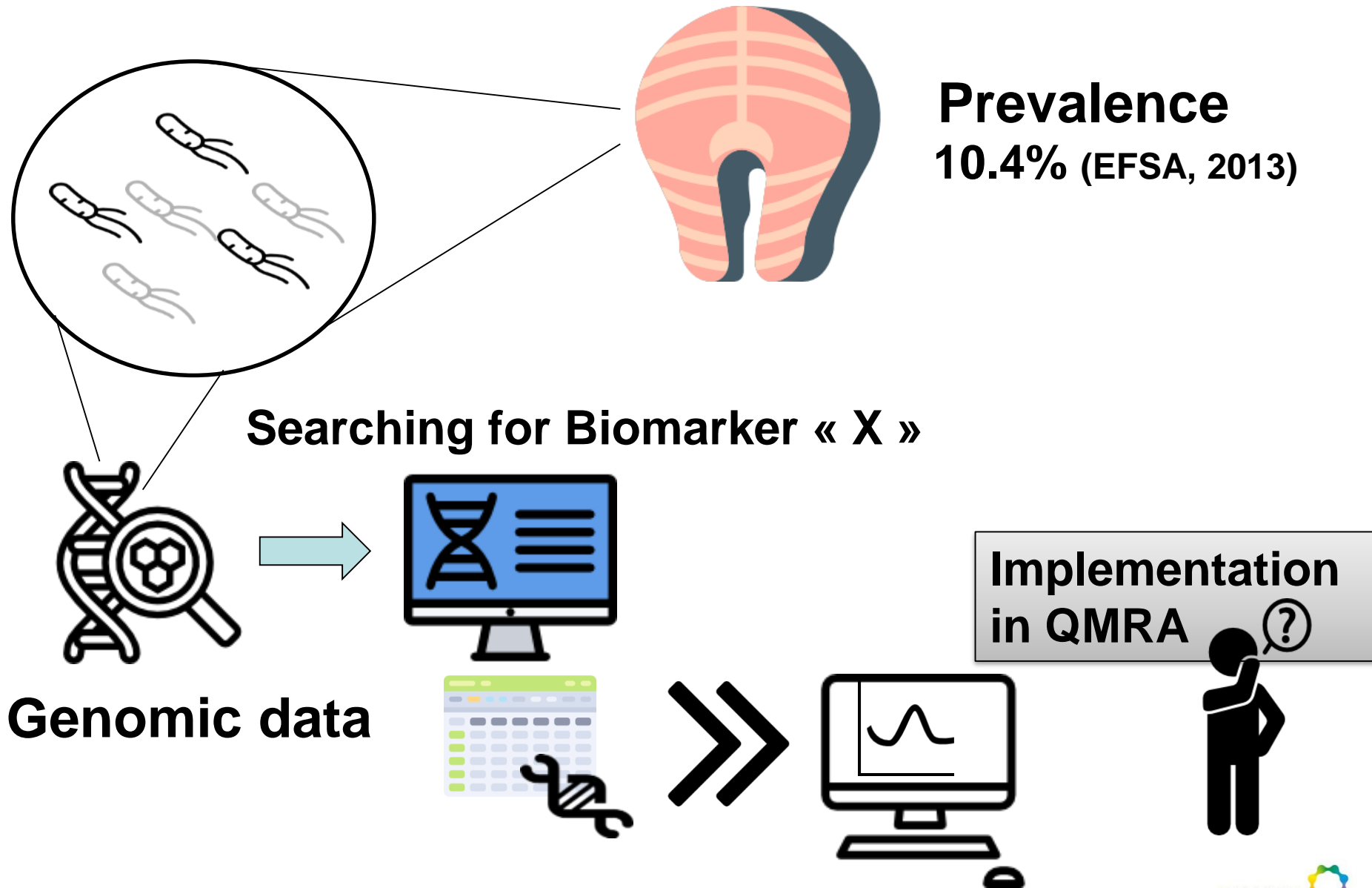
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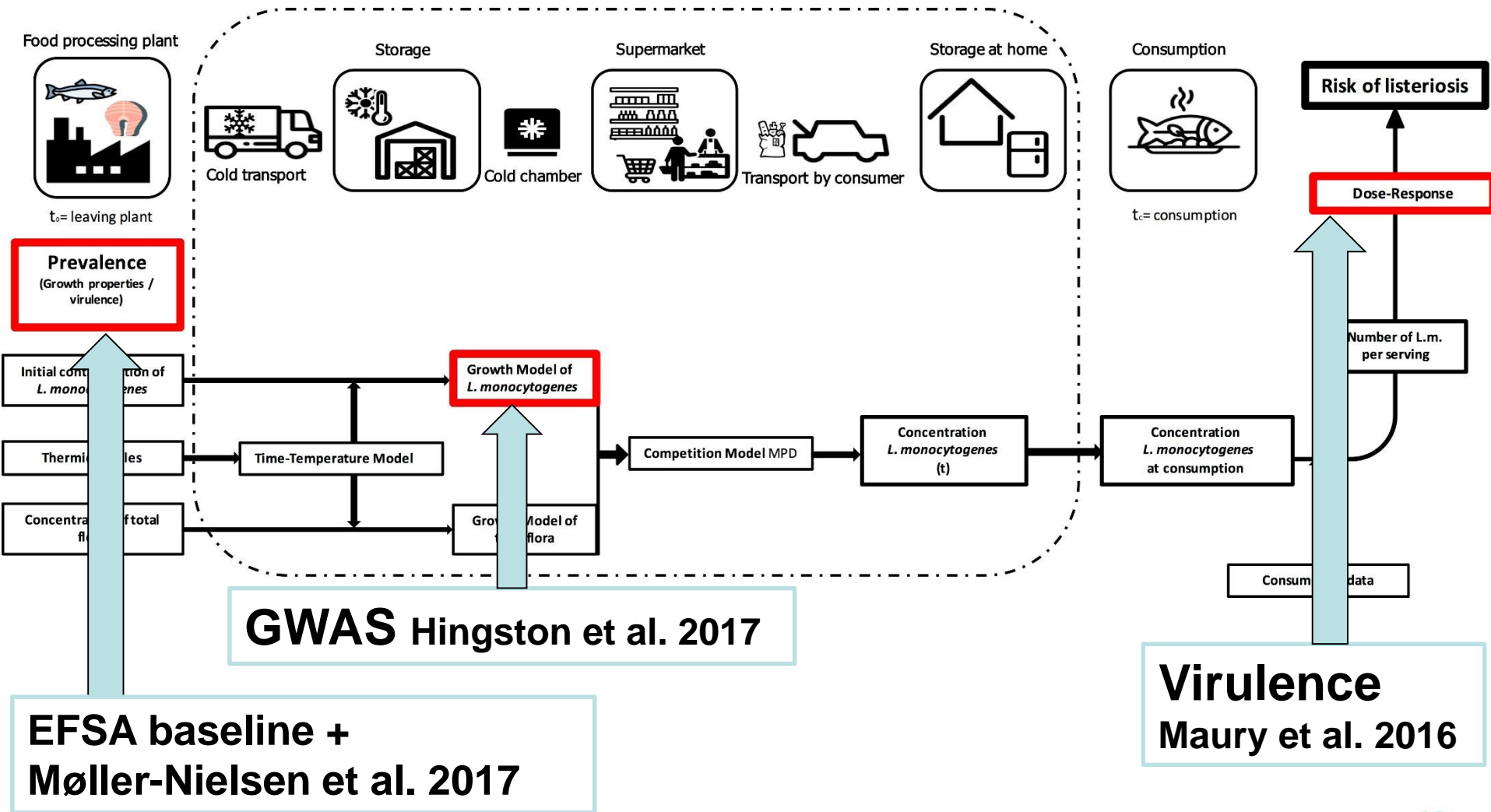
0



How to use genomic data ?

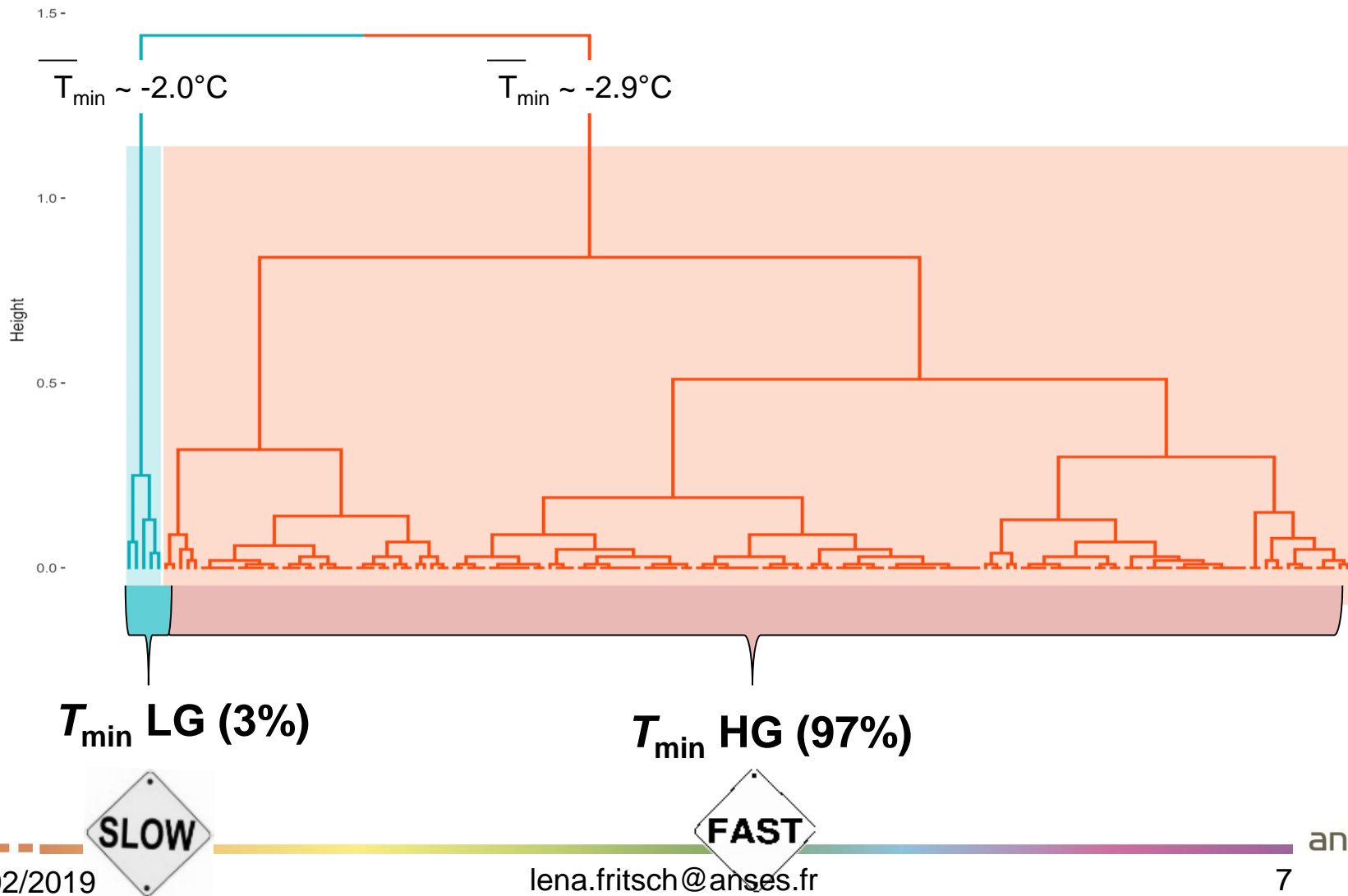


- Cold smoked salmon model (Pouillot et al., 2007, 2009)



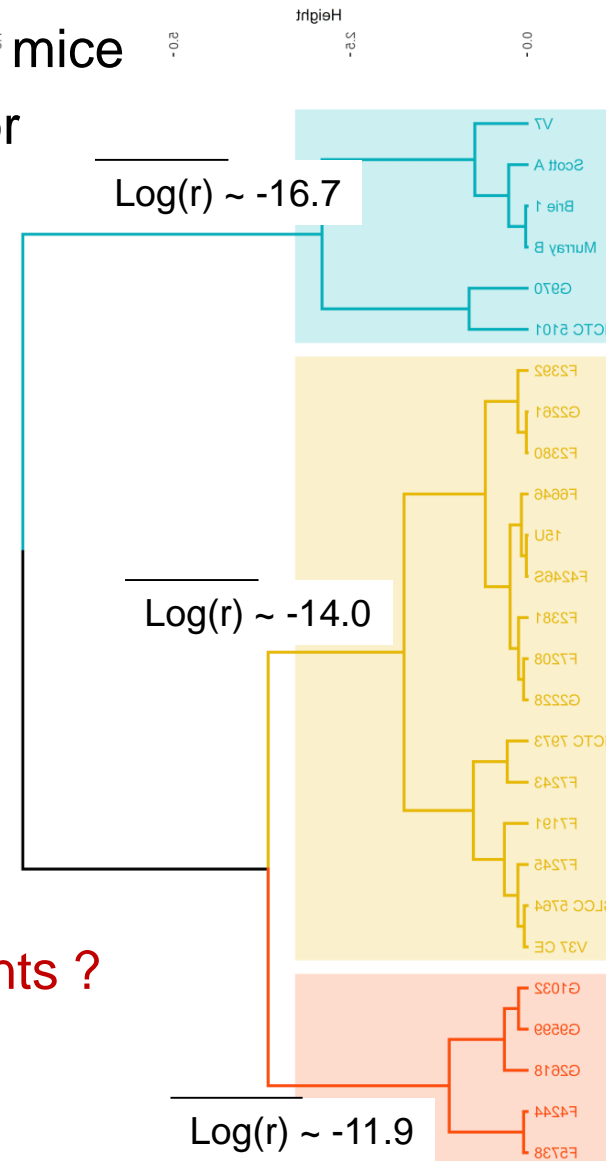
T_{\min} – 166 strains (Hingston et al. 2017)

- Biomarker as Genes, SNPs, ...
- Biomarker full length inlA → adaptation to cold temperature



Virulence – 26 strains (FDA/FSIS 2003)

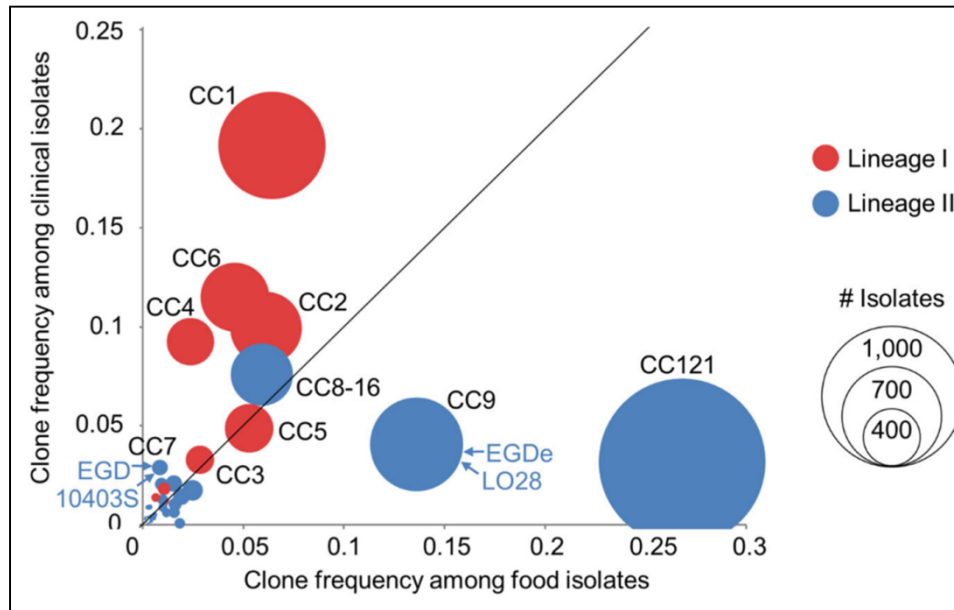
- **LD_{50}** intraperitoneal infection route in mice
- r -values deduced with a scaling factor
- $\text{Log}(r) = -\text{Log}(LD_{50}) - 8.79$



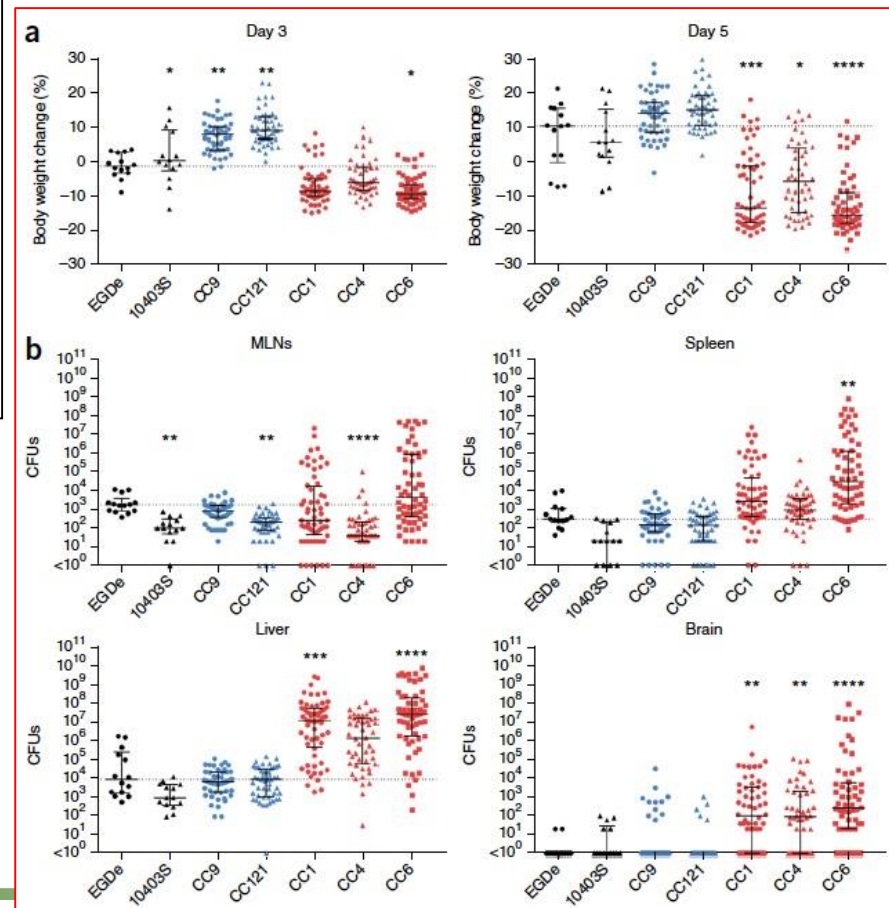
- Next steps
- Identification without mice experiments ?
- Distribution in cold smoked salmon ?

Virulence – MLST / clonal complexes (Maury et al. 2016)

- Hypo- and hypervirulent strains according to CC



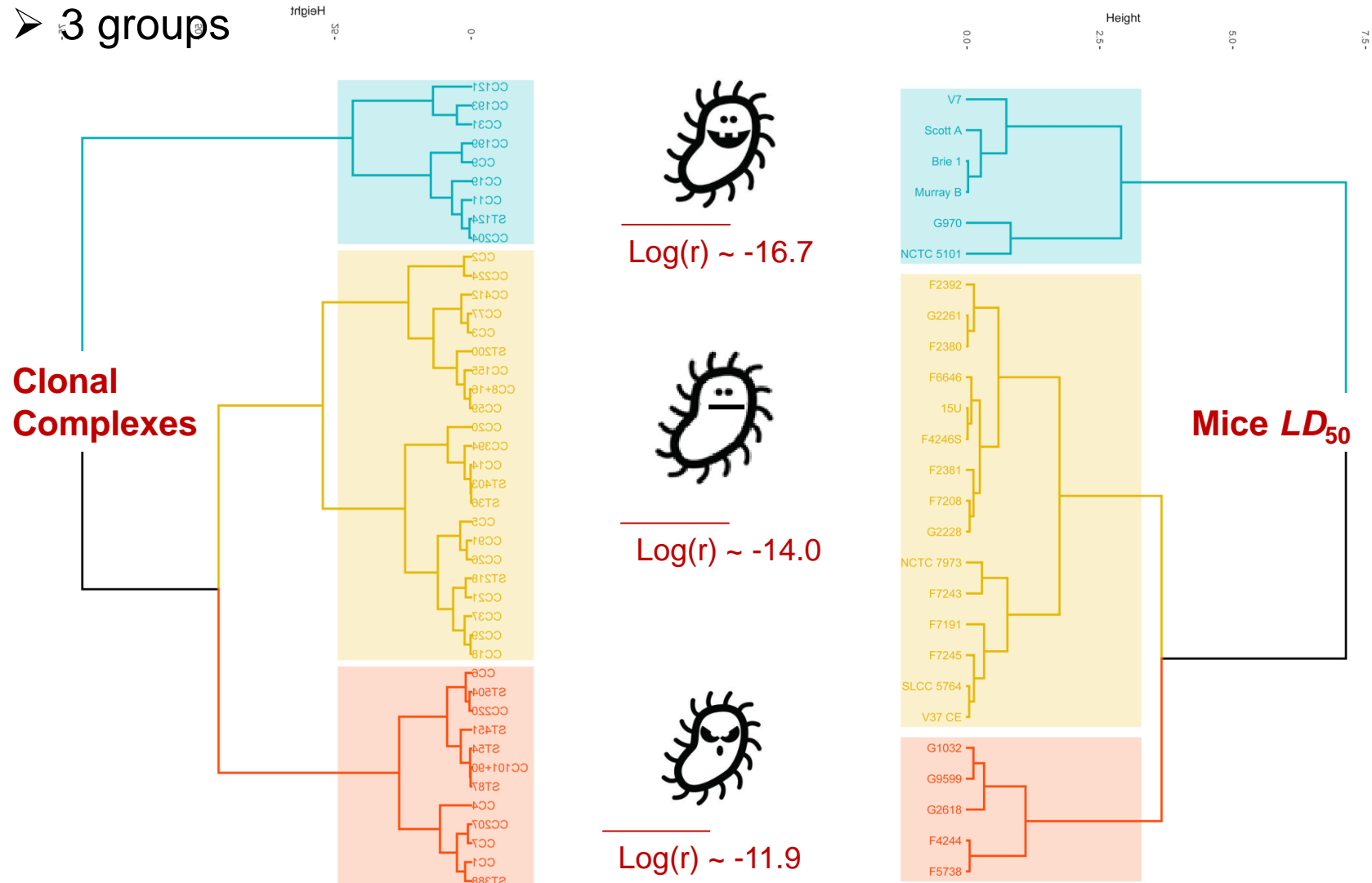
Humanized mice-model



Virulence – MLST / clonal complexes (Maury et al. 2016)

- **Relative clinical frequency** of CC assumed reflecting the virulence

➤ 3 groups



Distribution of CCs in cold smoked salmon (Møller-Nielsen et al. 2017)

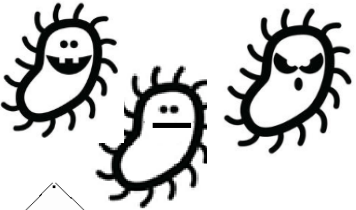
- 294 strains

CCs	Number of isolates	Total	Virulence group
CC1	1	37 (12.6%)	Hyper
CC2	4		
CC6	10		
CC7	8		
CC87	6		
CC101	7		
CC451	1		
CC3	9	105 (35.7%)	Medium
CC5	1		
CC8	49		
CC14	5		
CC20	4		
CC21	1		
CC59	6		
CC177	1		
CC155	24		
CC403	4		
new CC	1		
CC9	37	152 (51.7%)	Hypo
CC121	93		
CC19	3		
CC31	5		
CC193	4		
CC204	8		
ST124	2		
Total	294		

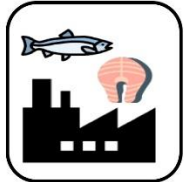
And now, let's run the QMRA models !



Prevalence 10.4%
(EFSA 2013)



Food processing plant



t_c = leaving plant

Prevalence
(Growth properties / virulence)



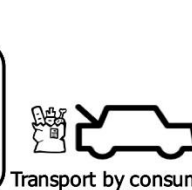
Cold transport



Storage



Supermarket



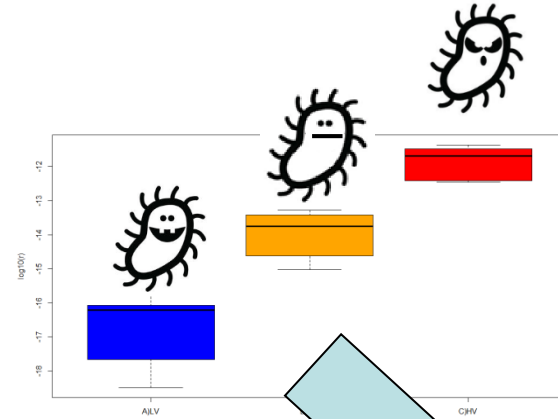
Transport by consumer



Storage at home



t_c = consumption



Risk of listeriosis

Dose-Response

Number of L.m. per serving

Consumption data

Initial contamination of *L. monocytogenes*

Thermic profiles

Concentration of total flora

Time-Temperature Model

Growth Model of *L. monocytogenes*

Growth Model of total flora

Competition Model MPD

Concentration *L. monocytogenes* (t)

Concentration *L. monocytogenes* at consumption

And now, let's run the QMRA models !



Prevalence
10.4%

Specific

Generic

(%)	Slow	Fast	Total
Hypervirulent	0.4	12.2	12.6
Medium virulence	1.1	34.6	35.7
Hypovirulent	1.6	50.1	51.8
Total	3.0	97.0	100

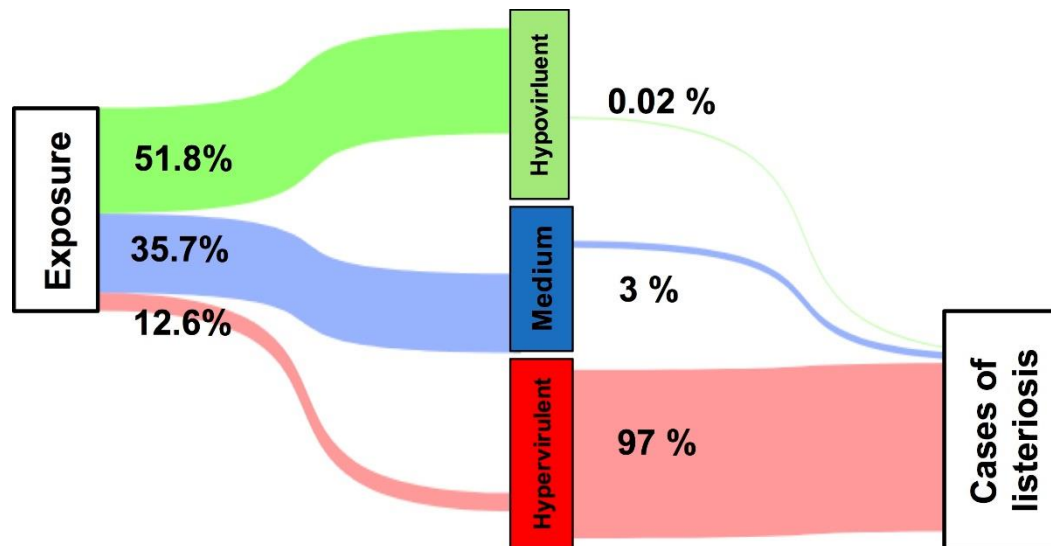
T_{\min} : 1 empirical distribution
of 166 values

r-values : 1 empirical
distribution of 26 values

T_{\min} : 2 distributions = slow, fast

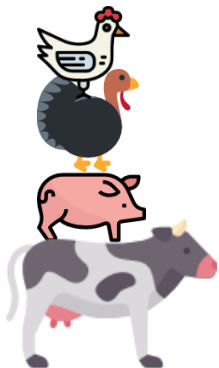
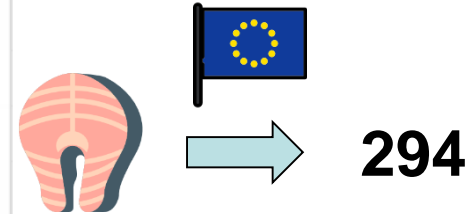
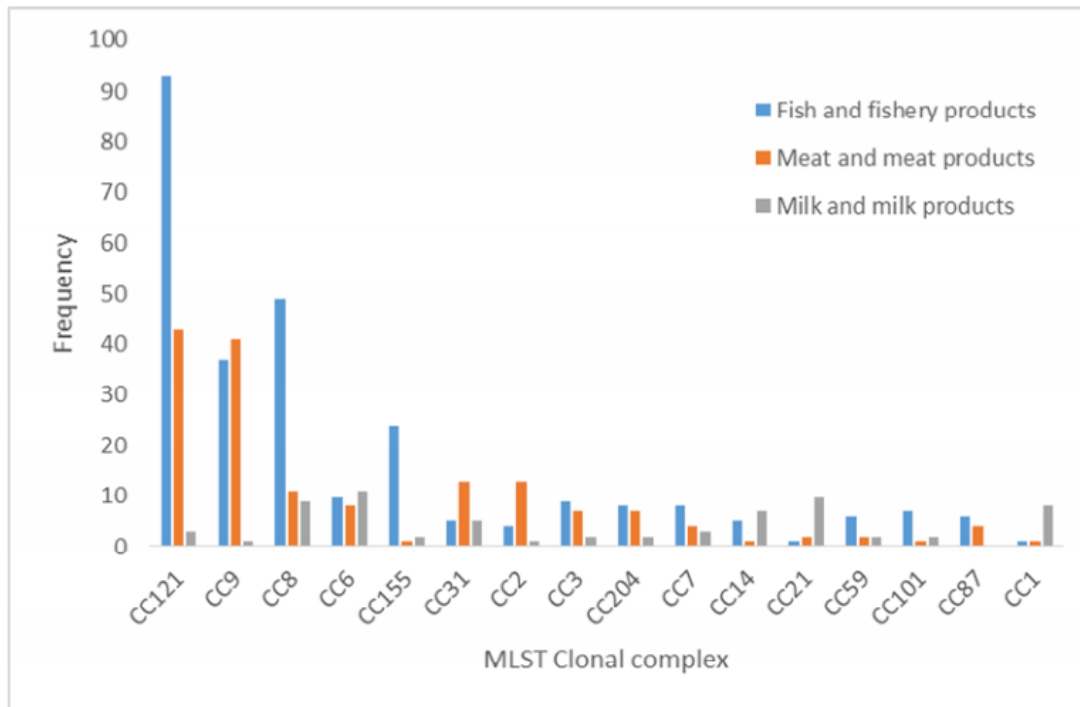
r-values : 3 distributions = hyper-,
medium, hypo-virulent

Conclusions



- Proof of concept (integration of WGS in risk assessment)
- Strong hypothesis: r -values \longleftrightarrow CCs \longleftrightarrow clinical frequency
- Important issue: consider the population structure of strains
- Near future \rightarrow Biomarkers
 \rightarrow Accessible data
- Adapting or refinement of intervention strategies (hypervirulent strains ?)

Future prospects: What about RTE meat products ?



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Thank you for your attention !

Many thanks to

Jean-Christophe Augustin

Laurent Guillier



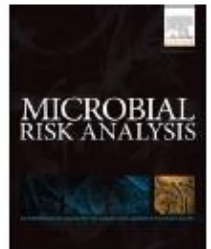
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Full length article

Next generation quantitative microbiological risk assessment: Refinement of the cold smoked salmon-related listeriosis risk model by integrating genomic data



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