

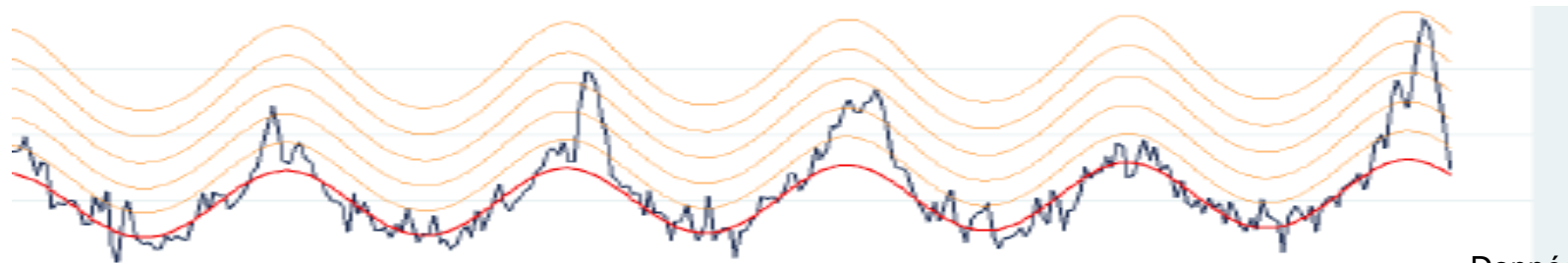
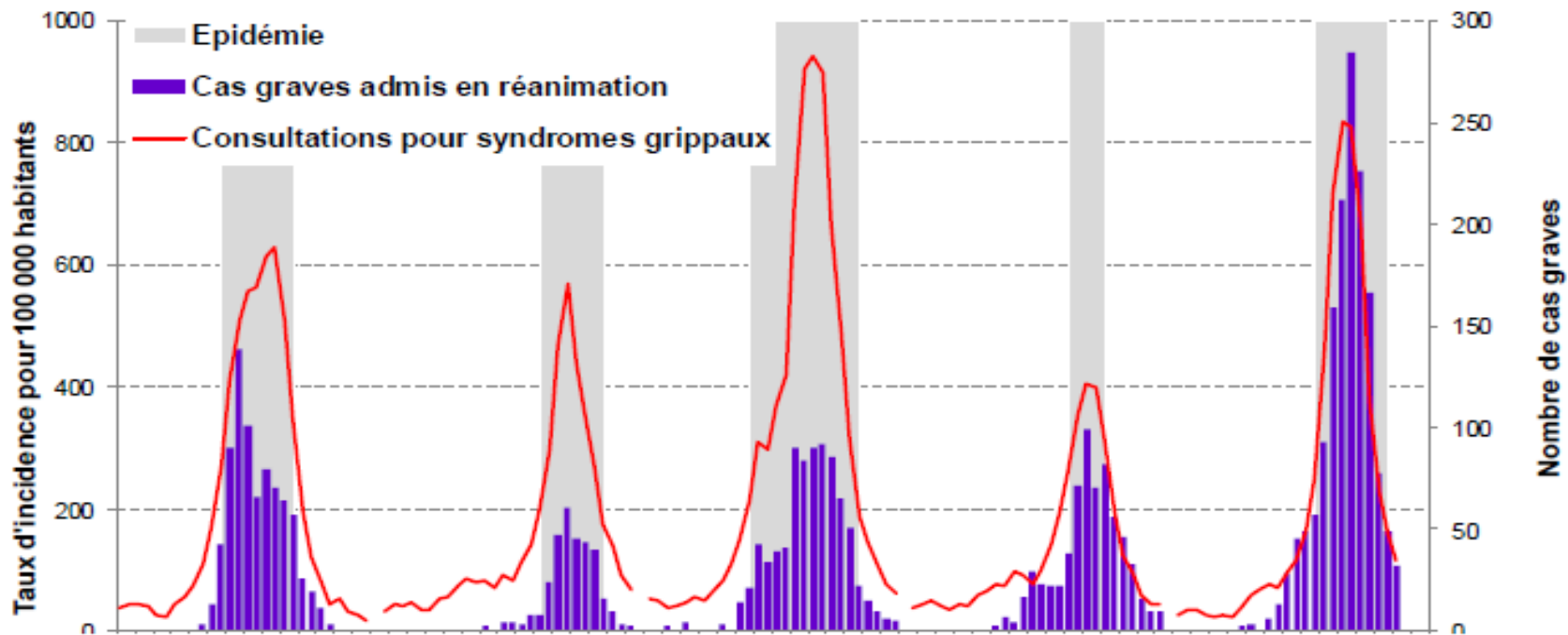


Comment prédire l'évolution des virus influenza?

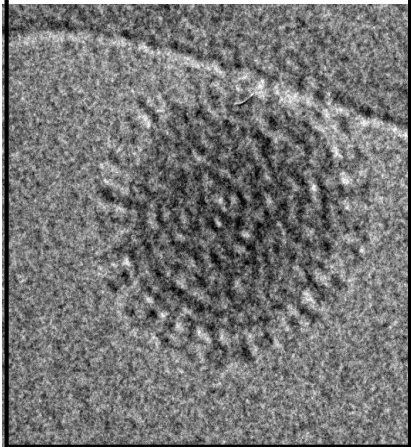
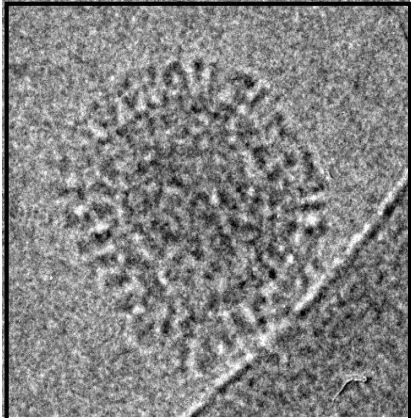
Bruno LINA

CNR des virus influenzae, HCL,
Virpath, CIRI, U1111, UMR 5308, ENS, UCBL,
Lyon, France

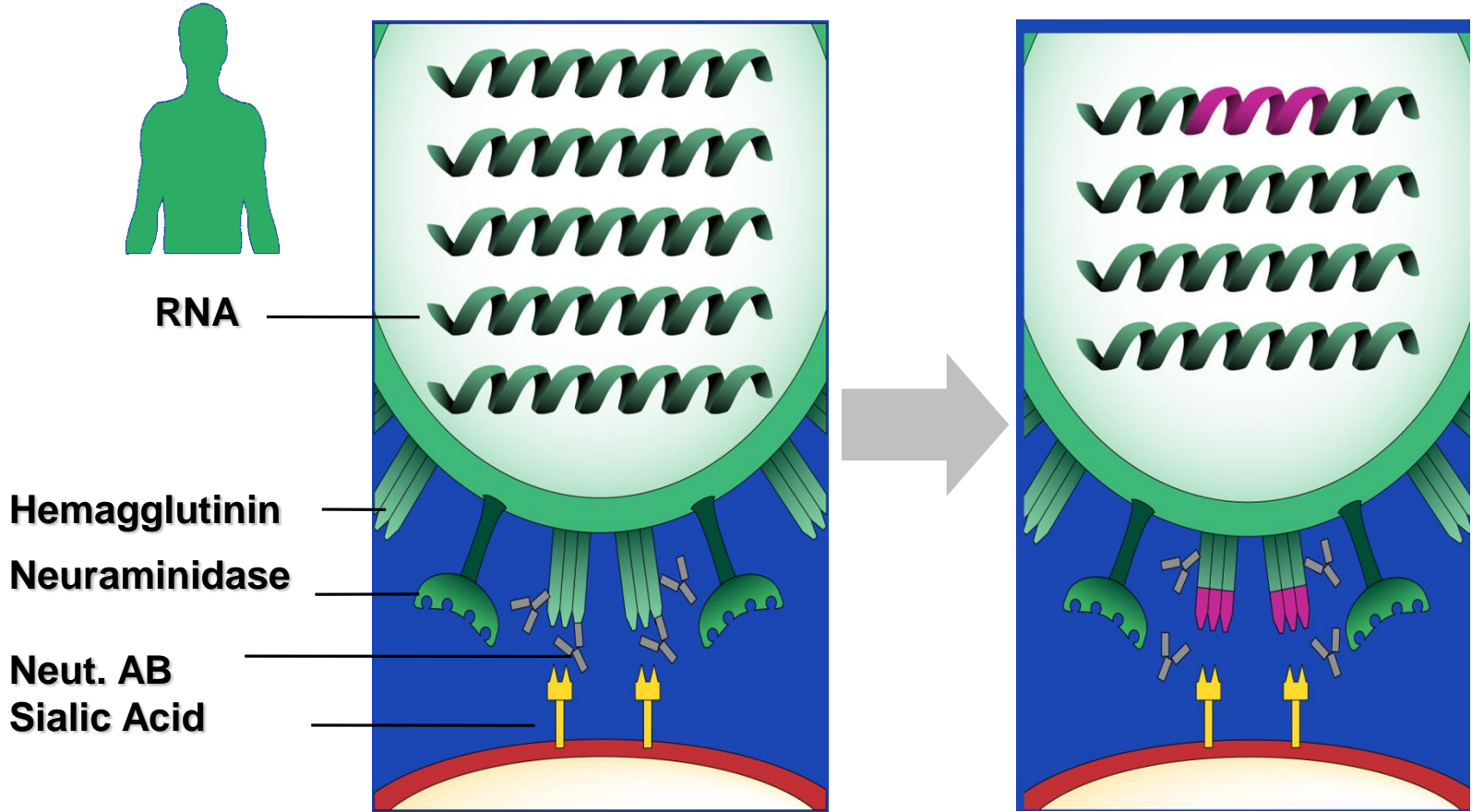
La grippe une maladie a impact variable



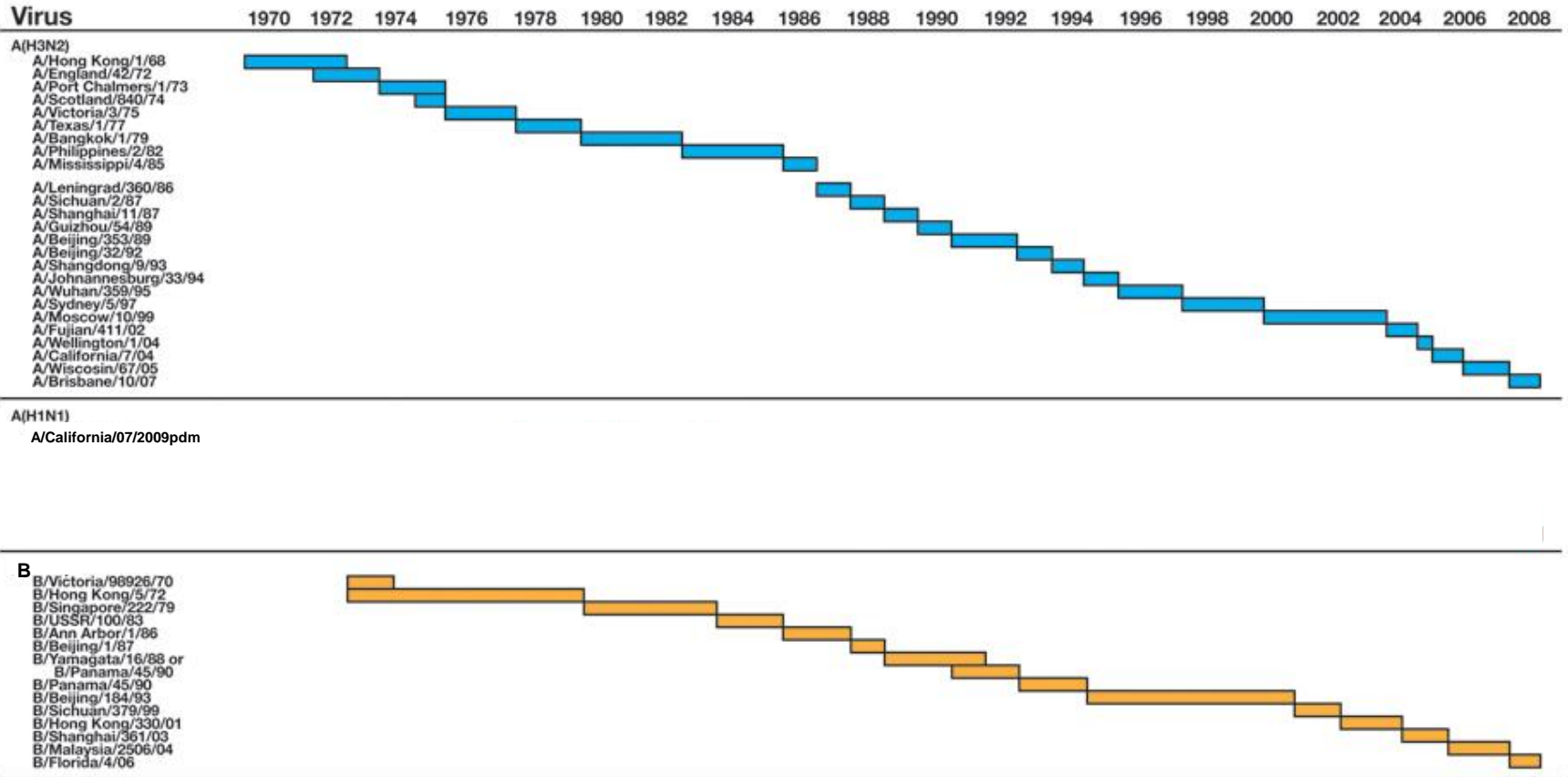
Les fautifs : le virus et l'hôte



Conséquences : le glissement antigénique

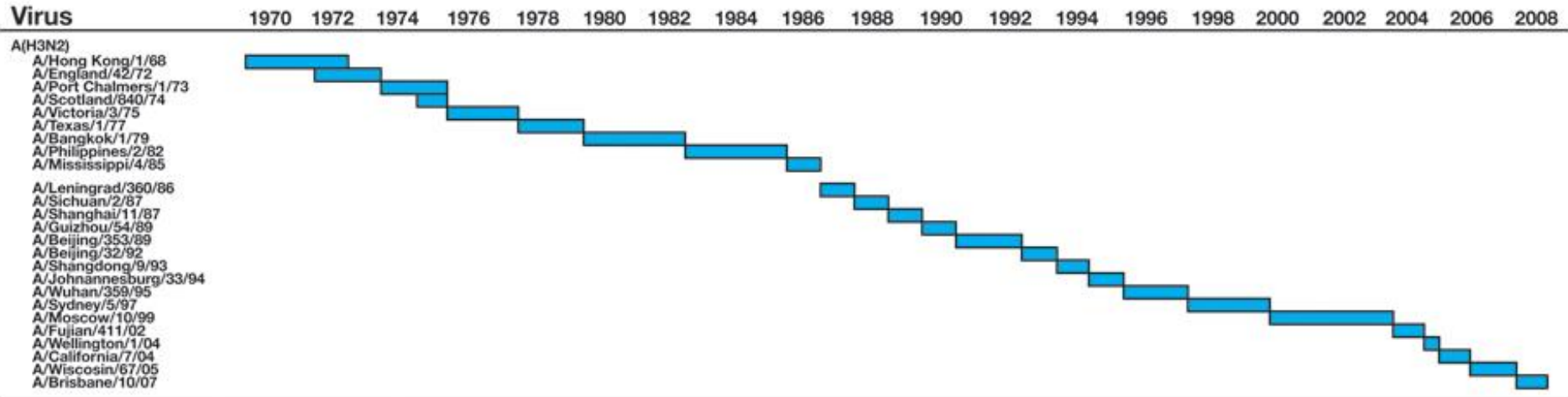


Conduisant au changement des prototypes vaccinaux

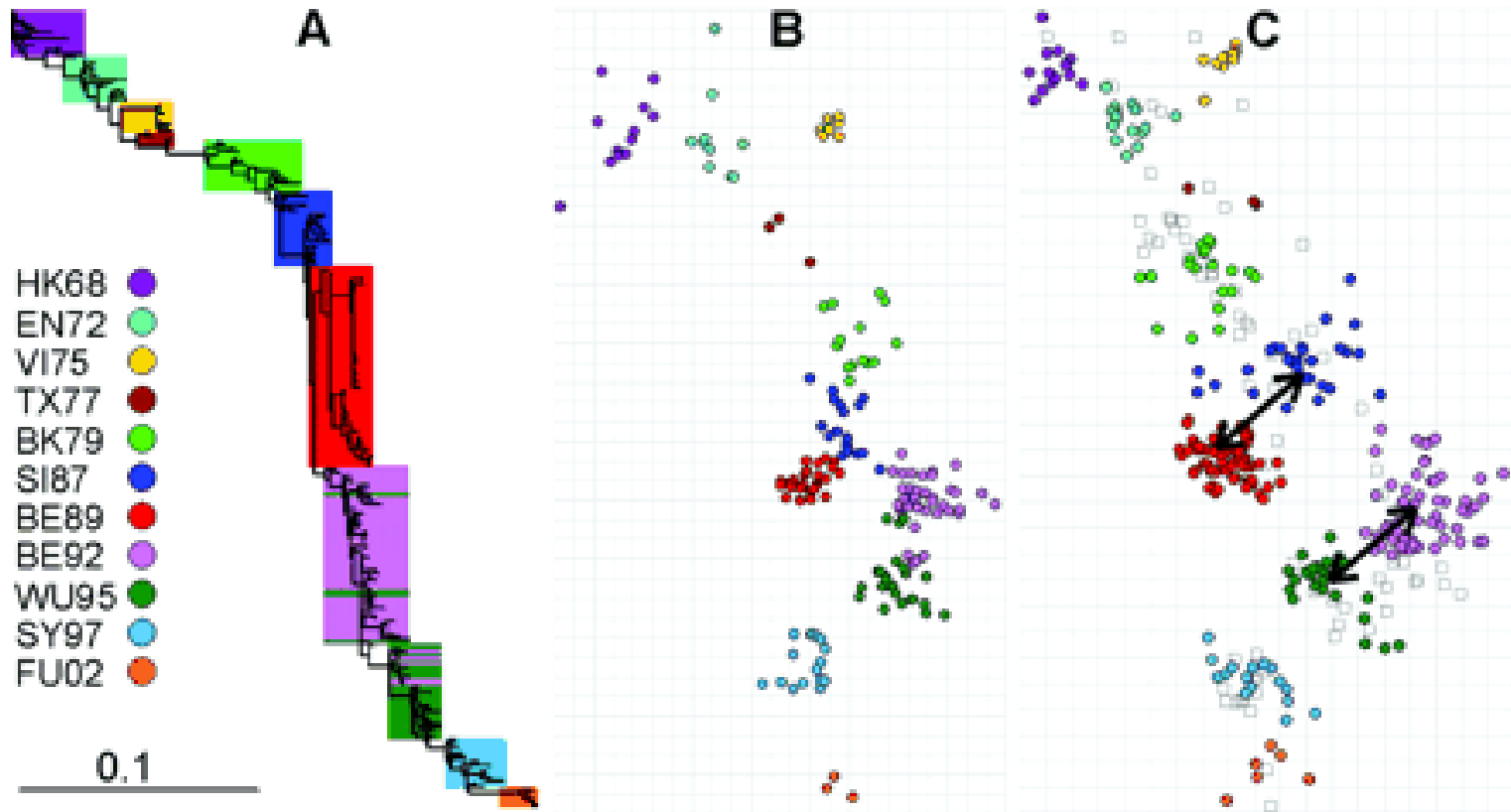


Il existe des outils pour
surveiller et prédire l'évolution
des virus influenza

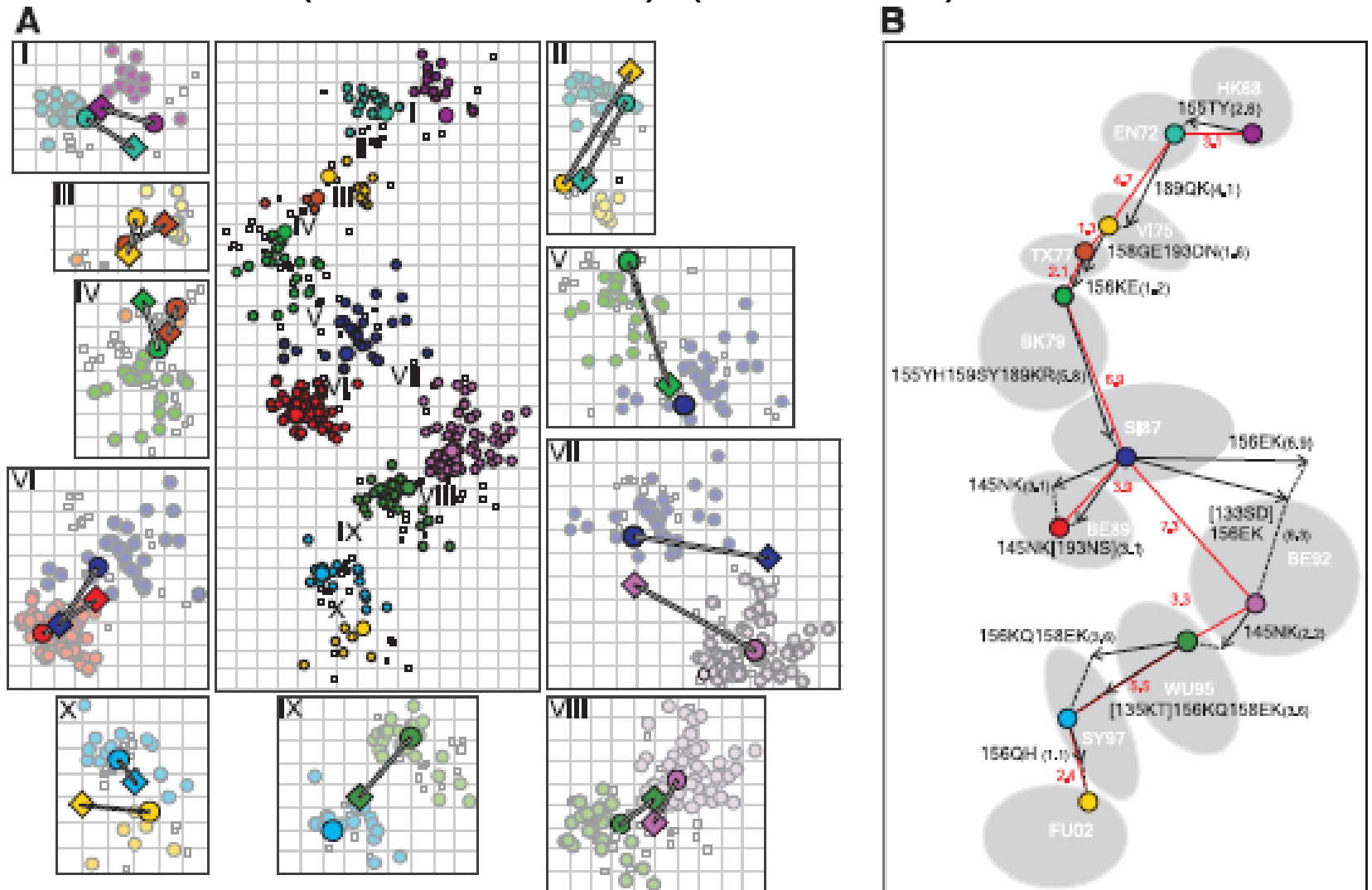
Evolution de H3N2 (1968 - 2009)

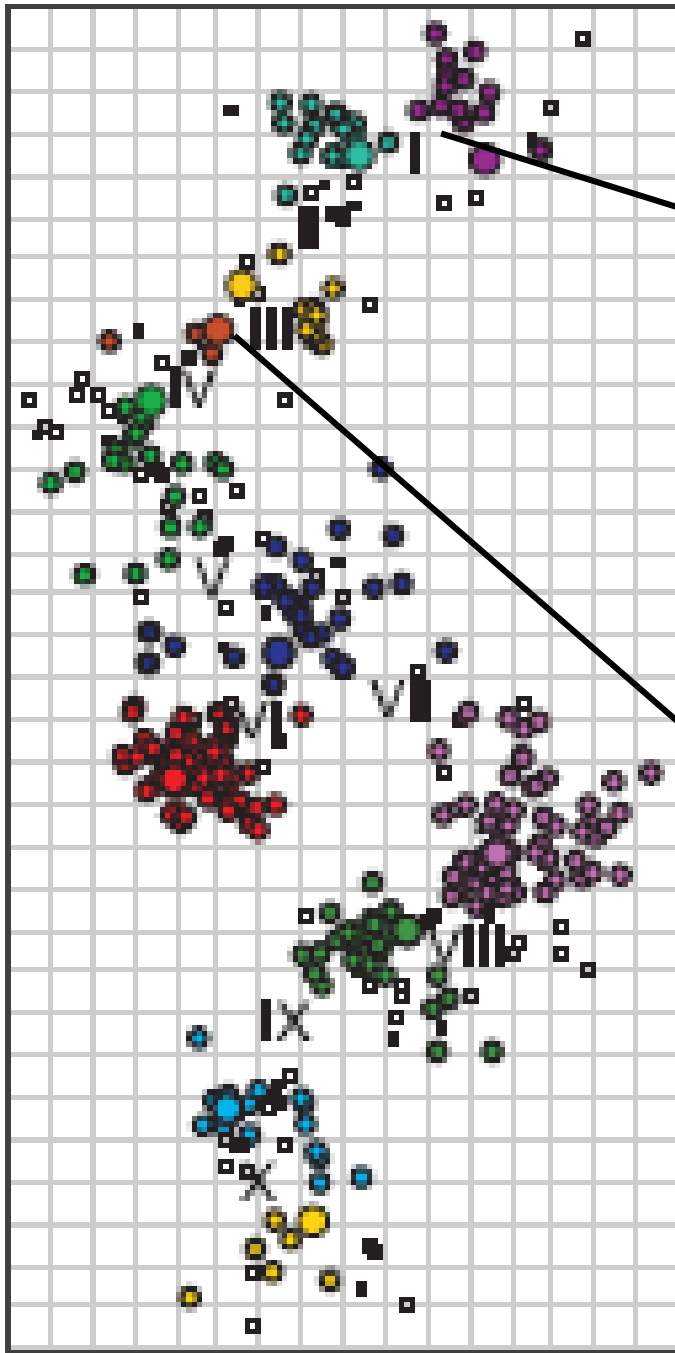


Antigenic distances Model (D Smith 2003)



L'évolution des H3N2 cartographiée et dissequée (1968 - 2002) (Fouchier)

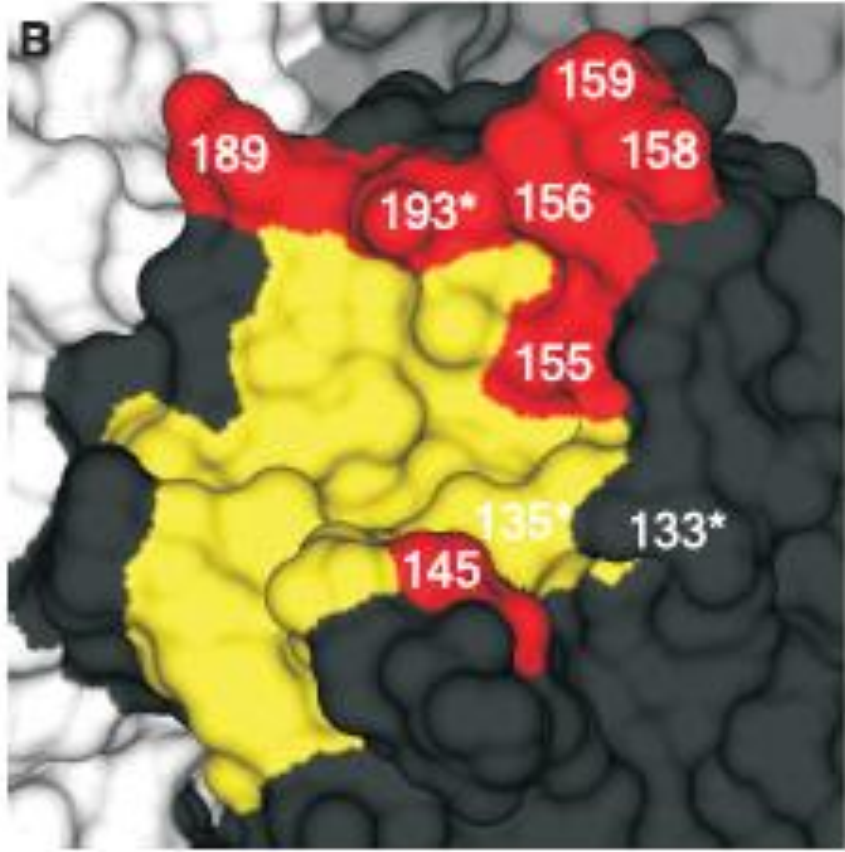
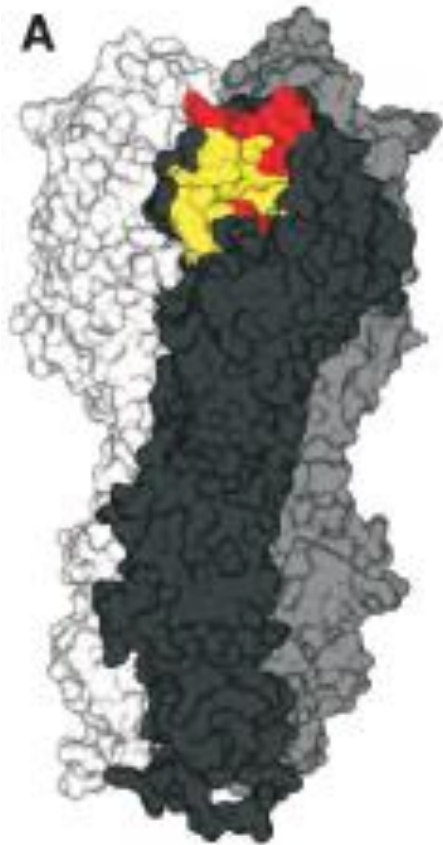




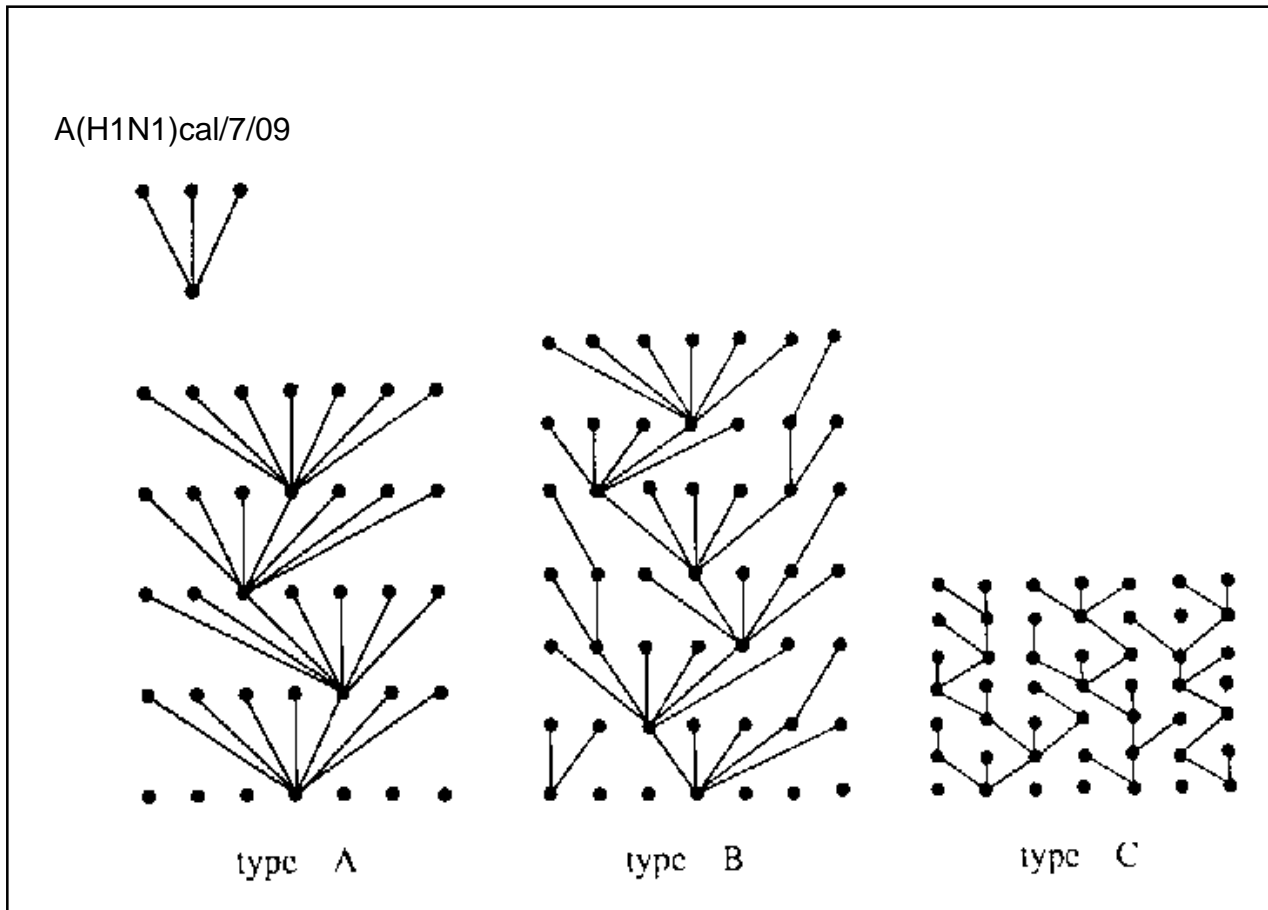
189KQ

189QK

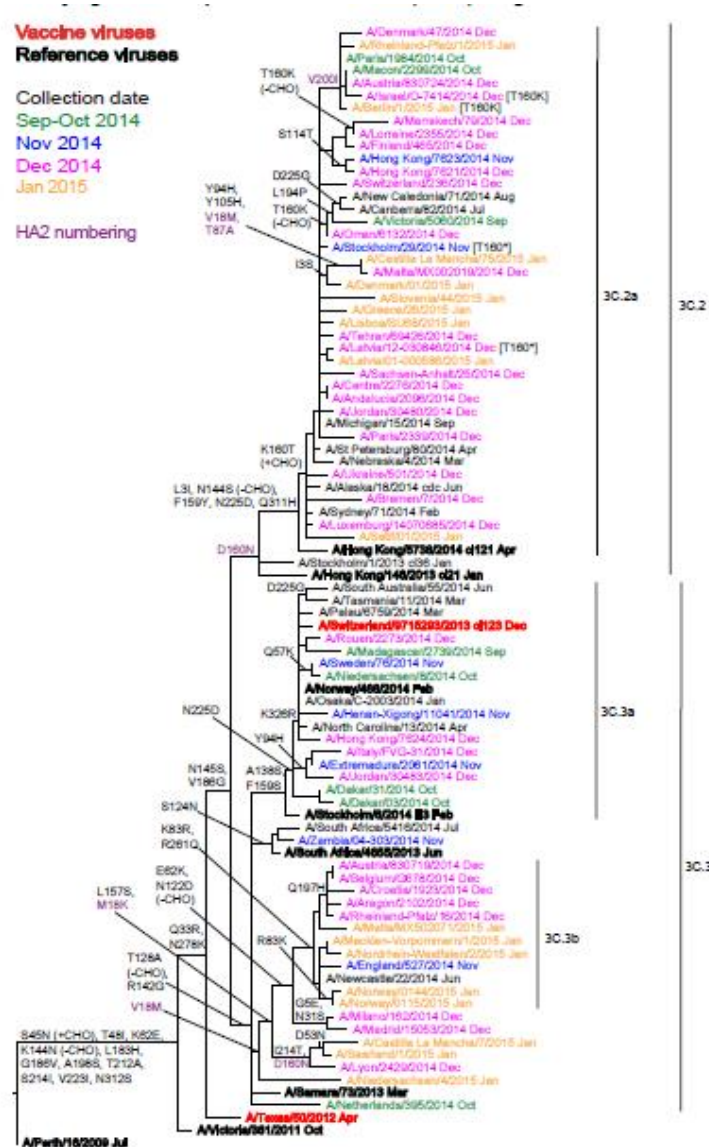
Les positions clefs des variations H3N2 majeures



Les virus dérivent en fonction de leur variations antigénique et de leur fitness

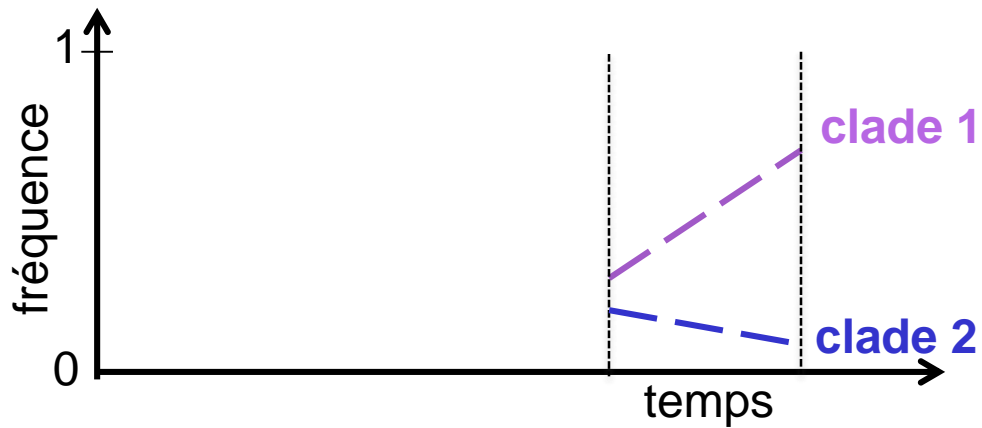
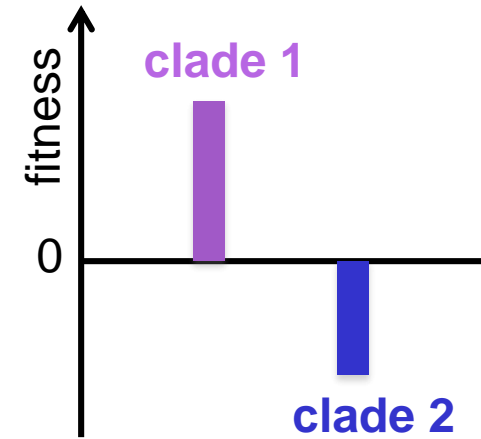
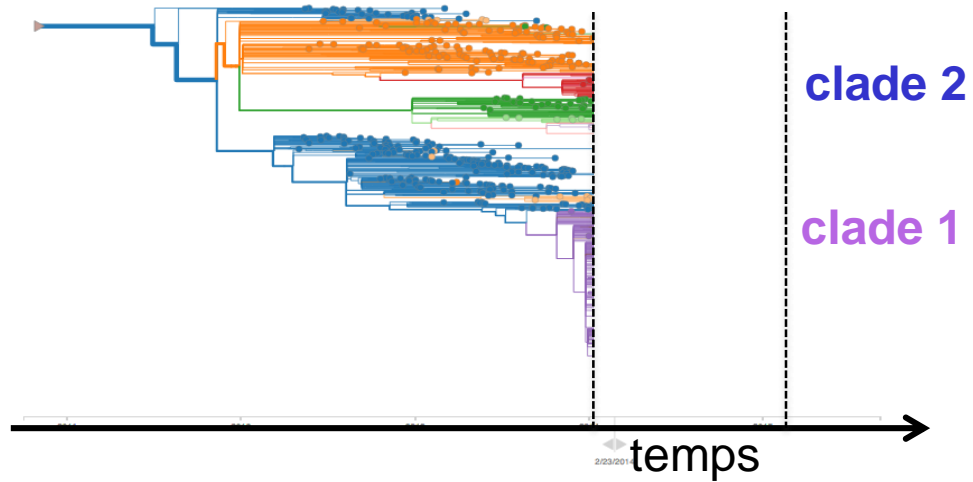


Les informations disponibles pour étudier les virus



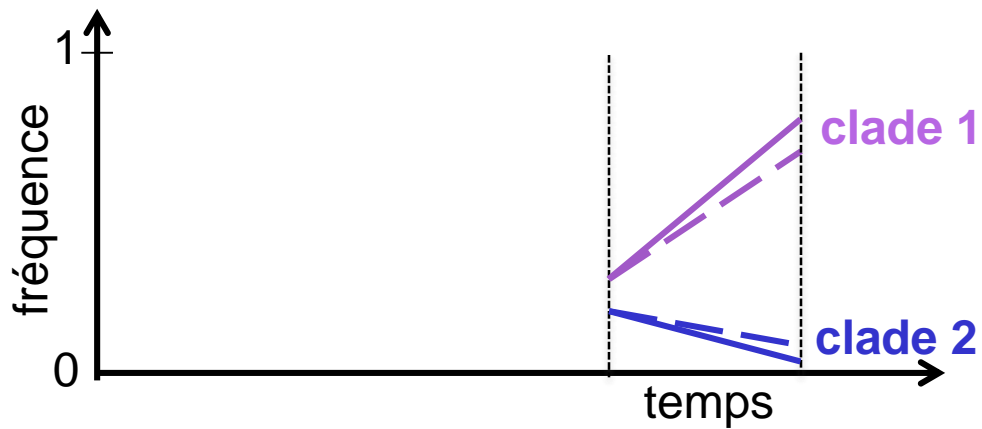
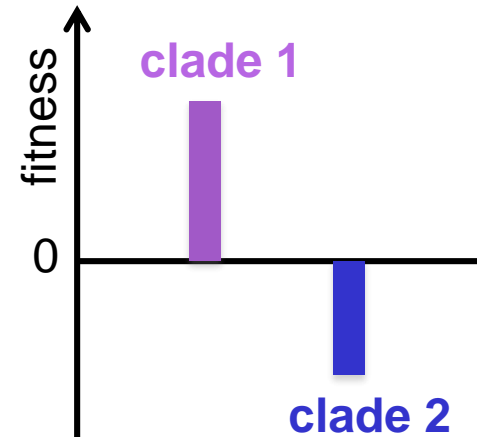
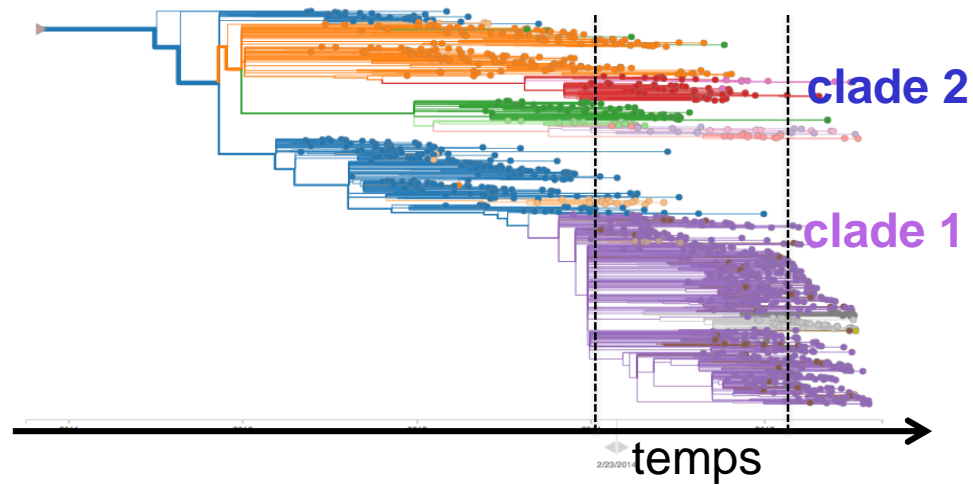
Comment réaliser une étude de fitness ?

Marta Luksza & Michael Lässig



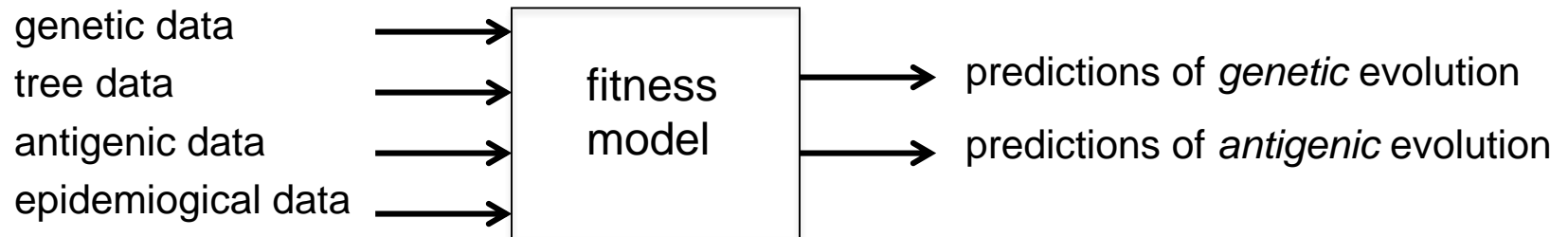
Les données fournies en temps réel permettent de mieux estimer

Marta Luksza & Michael Lässig



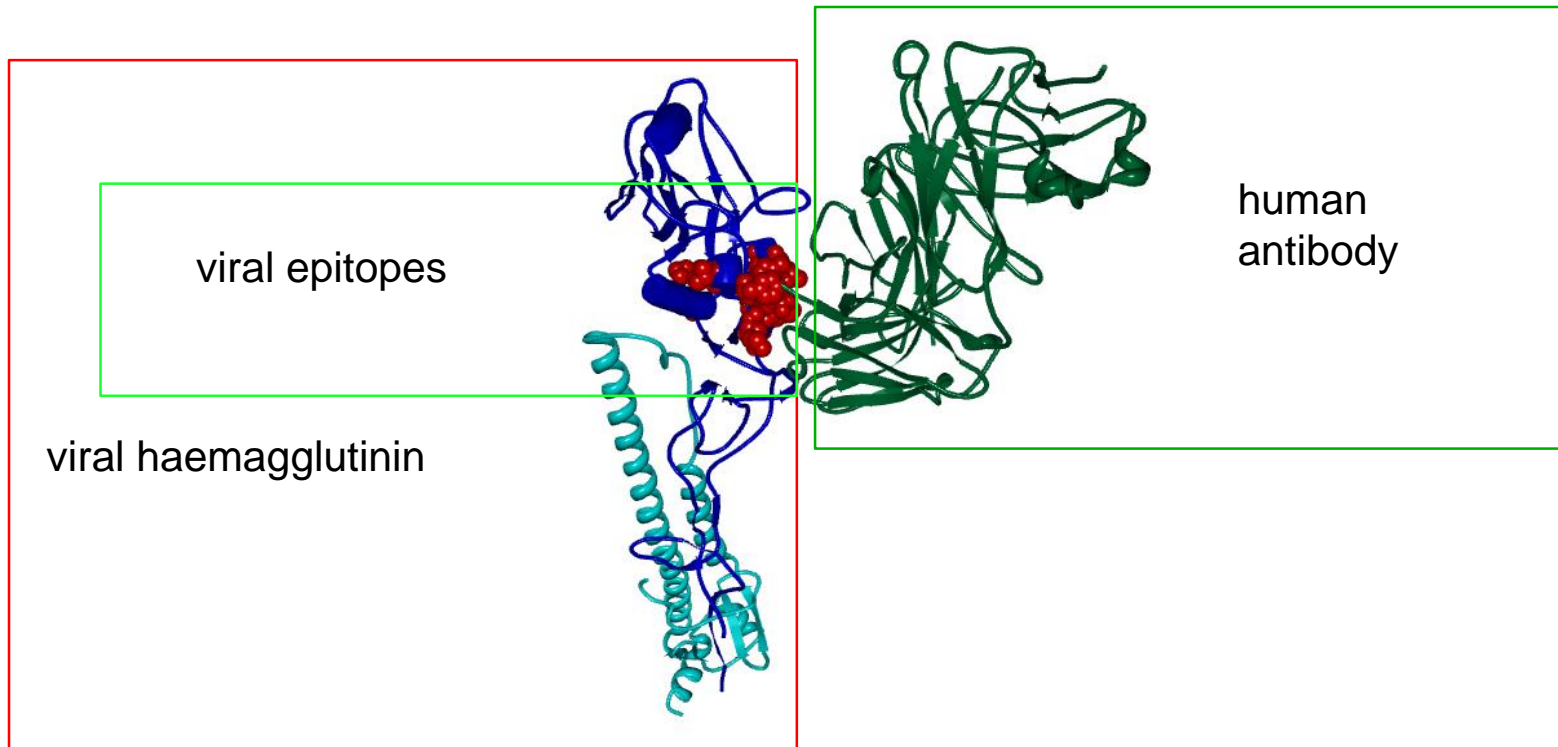
Donc un modele d'étude de fitness doit intégrer de nombreuses données

Fitness models integrate data into predictions



1. What is a fitness model?
- 2. Which data enter model predictions?**

Quelles sont les données nécessaires pour réaliser cette étude?

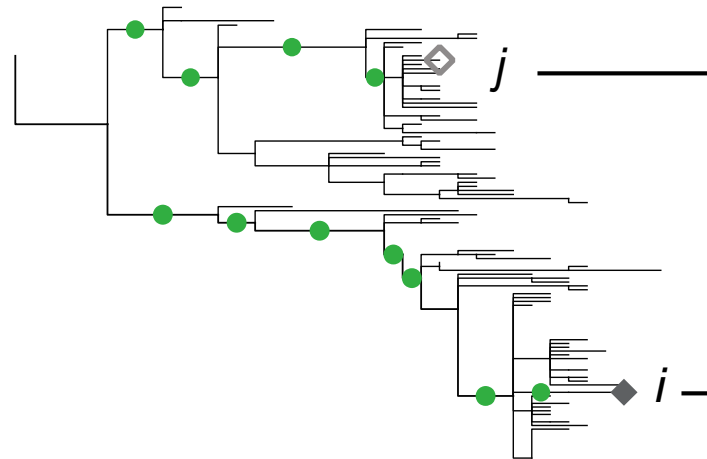


Données utilisées pour les études de fitness

Les données phylogénétiques des HA

non-epitope mutations:
decrease protein stability
→ *decrease fitness*

epitope mutations:
decrease binding affinity
→ *increase fitness*

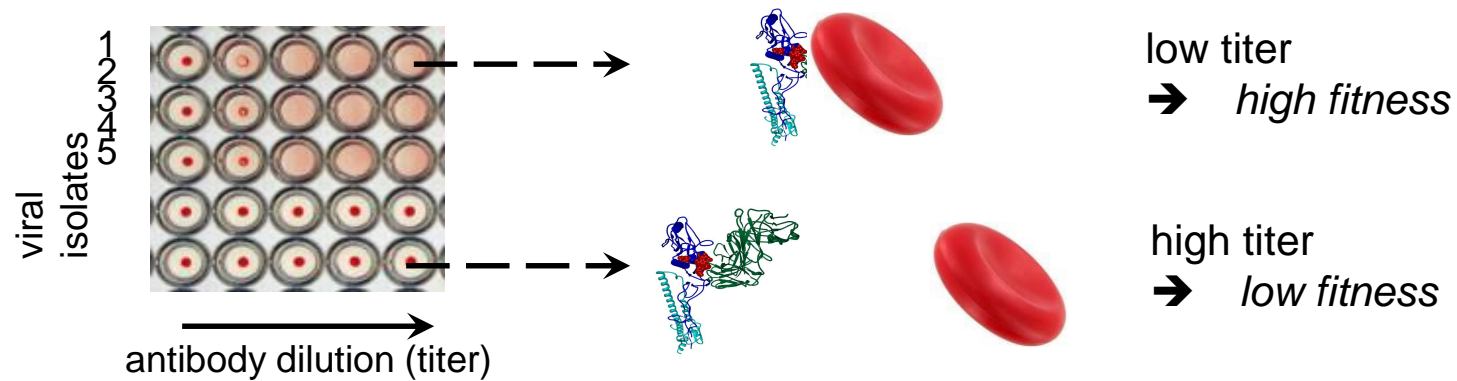


[M. Luksza and M.L. 2014]

Données utilisées pour les études de fitness

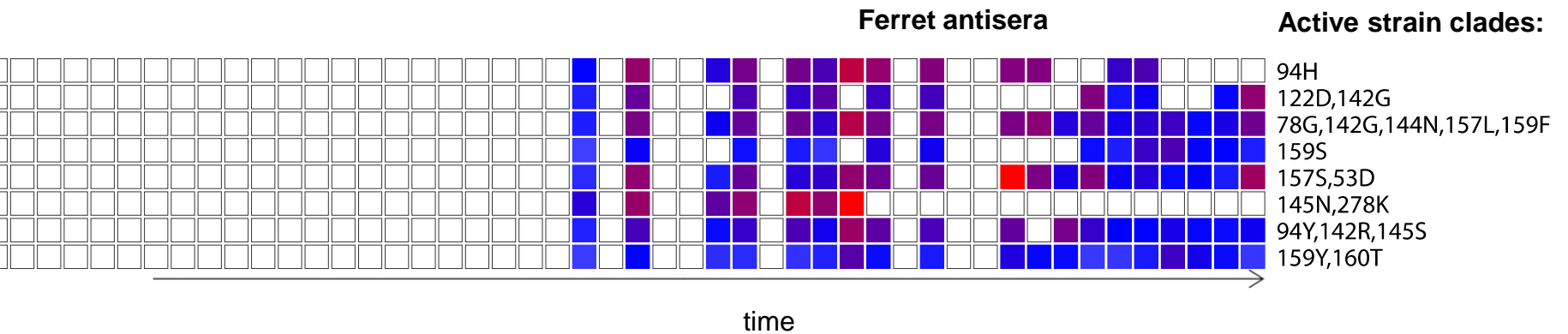
Données Antigéniques

HI or neutralization tests measure **binding affinity** between virus and antibodies.



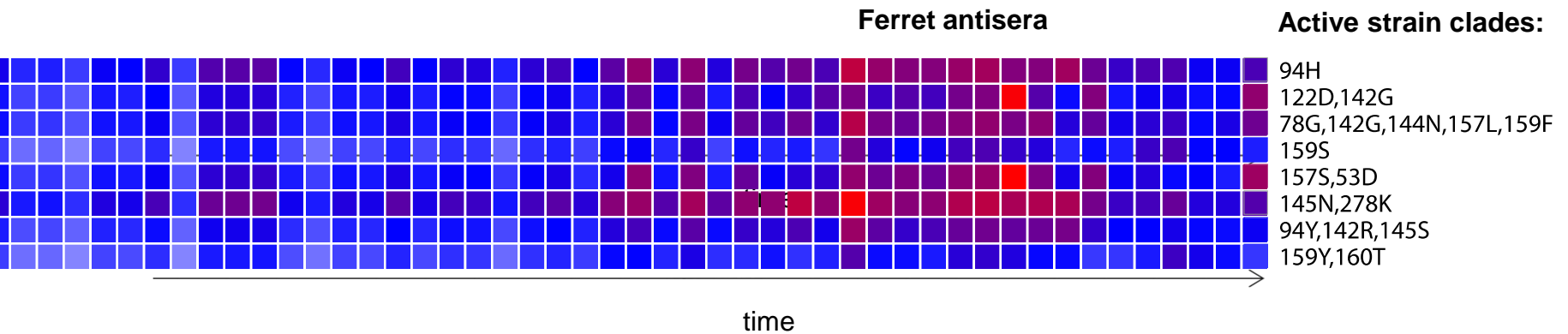
Données utilisées pour les études de fitness

Intégrer les données manquantes



Données utilisées pour les études de fitness

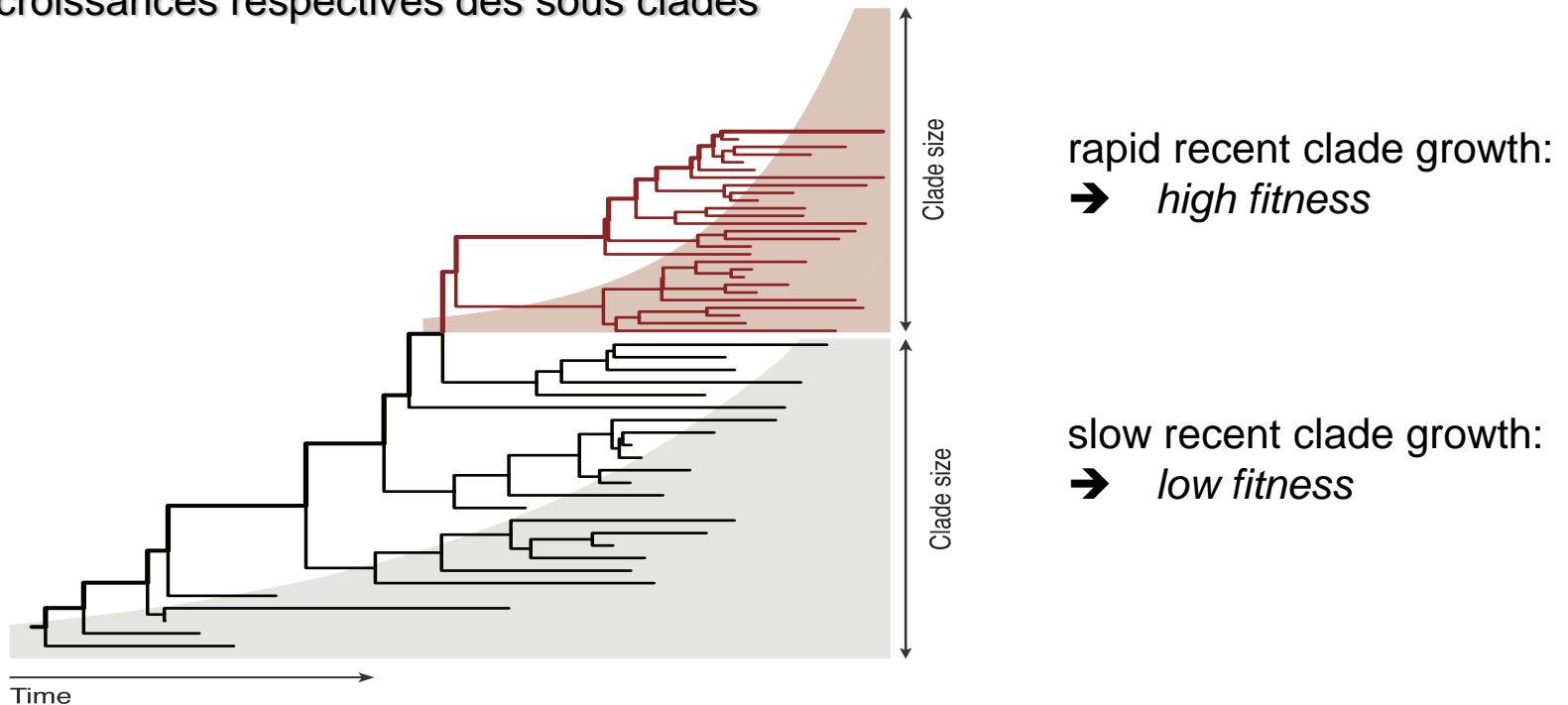
Qui parfois arrive a posteriori



Données utilisées pour les études de fitness

Tree-based fitness inference

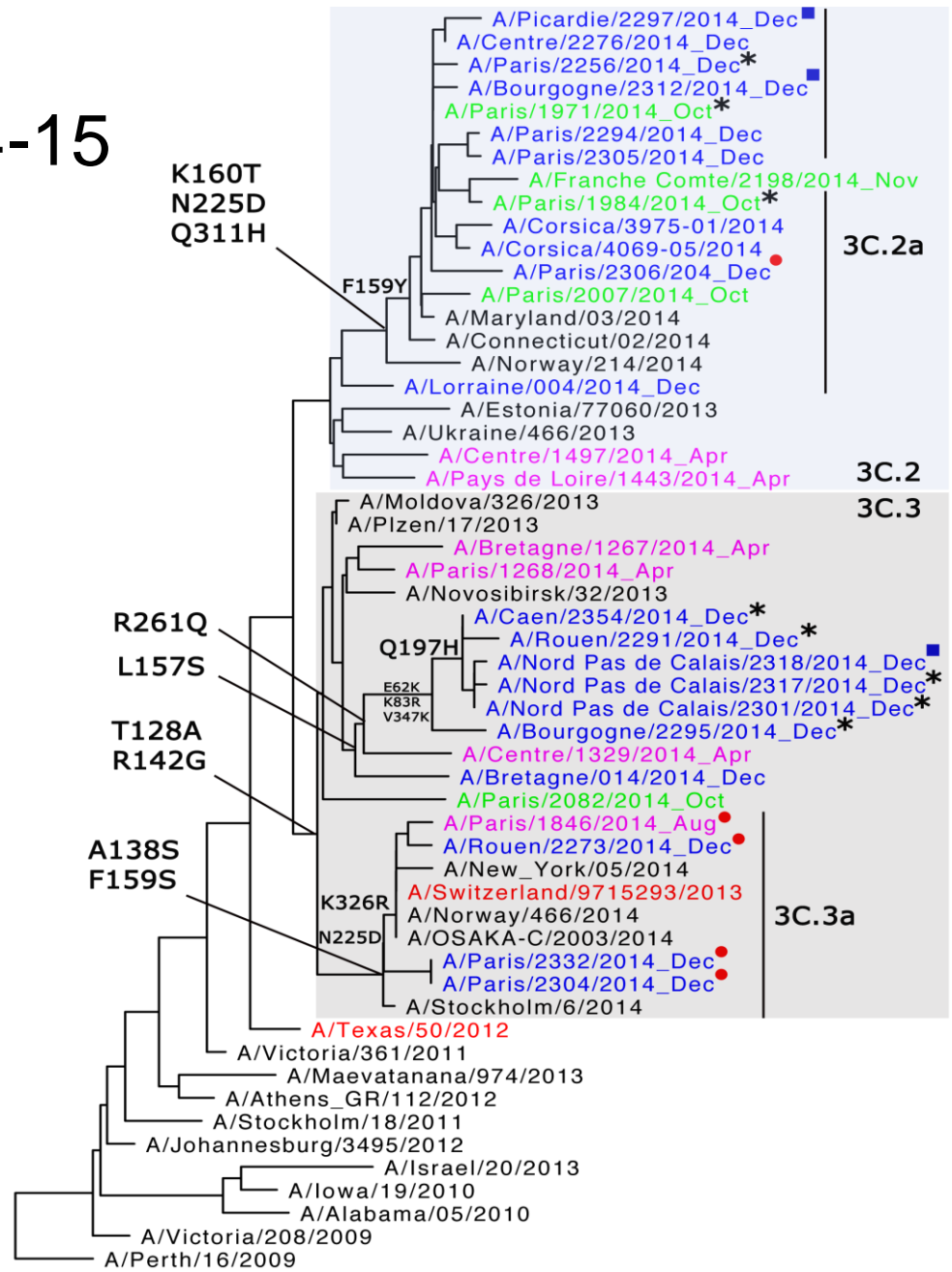
Utiliser des analyses phyogénétiques intégrant les évolutions génétiques et leur rapidité d'émergence pour étudier les croissances respectives des sous clades



[M. Luksza and M.L. 2014; R. Neher et al. 2014]

Mise en œuvre : 2014-15

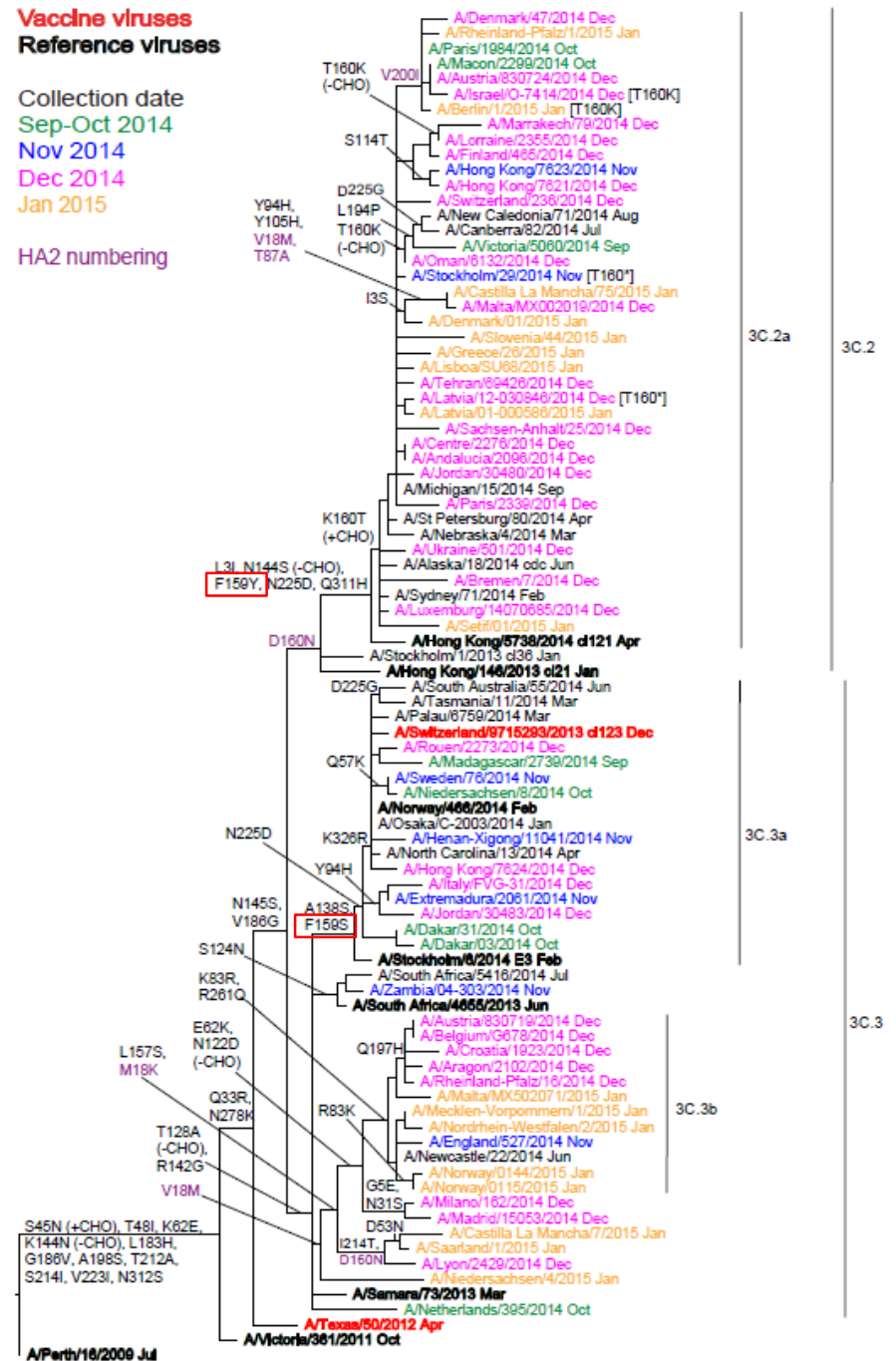
- switzerland –like
- ★ Texas-like
- indeterminates



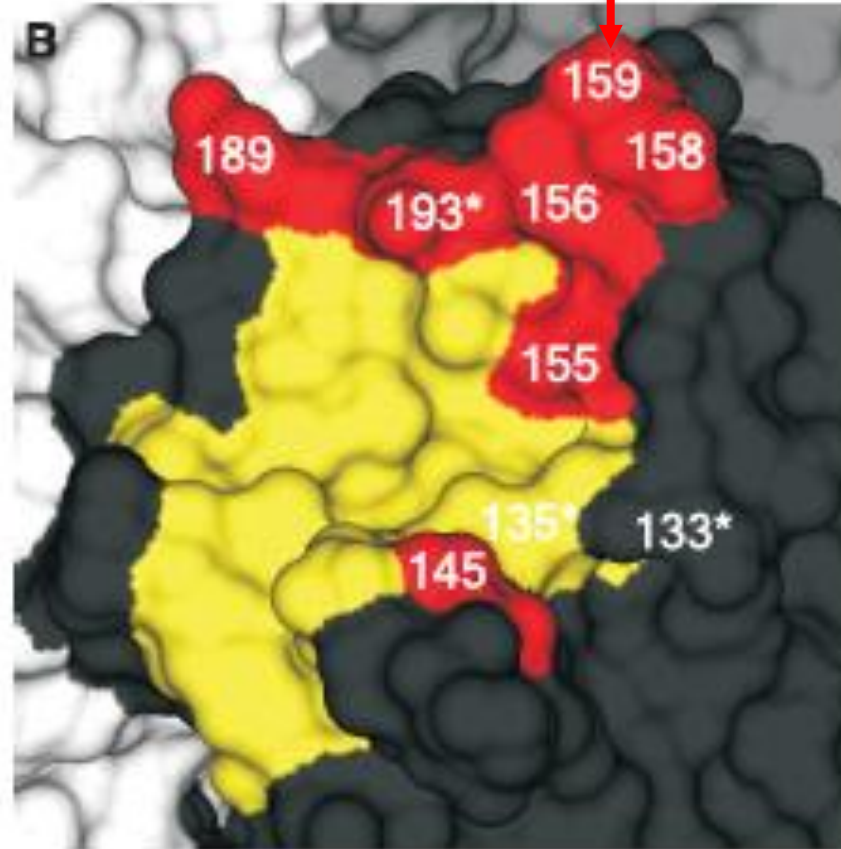
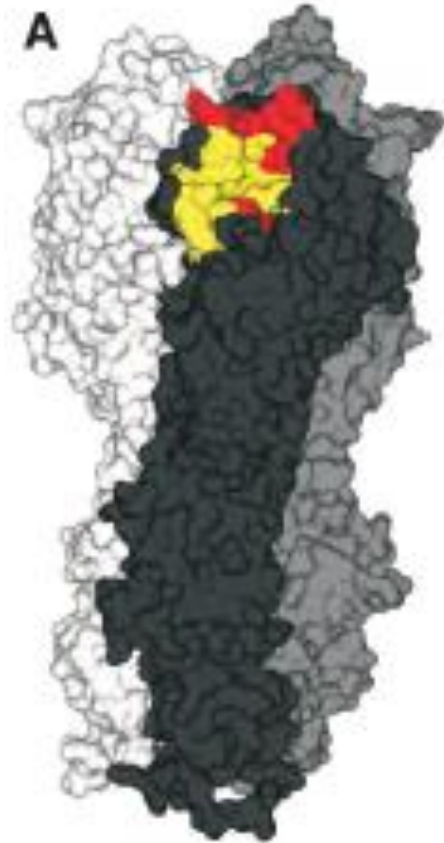
0.0030

2014-2015

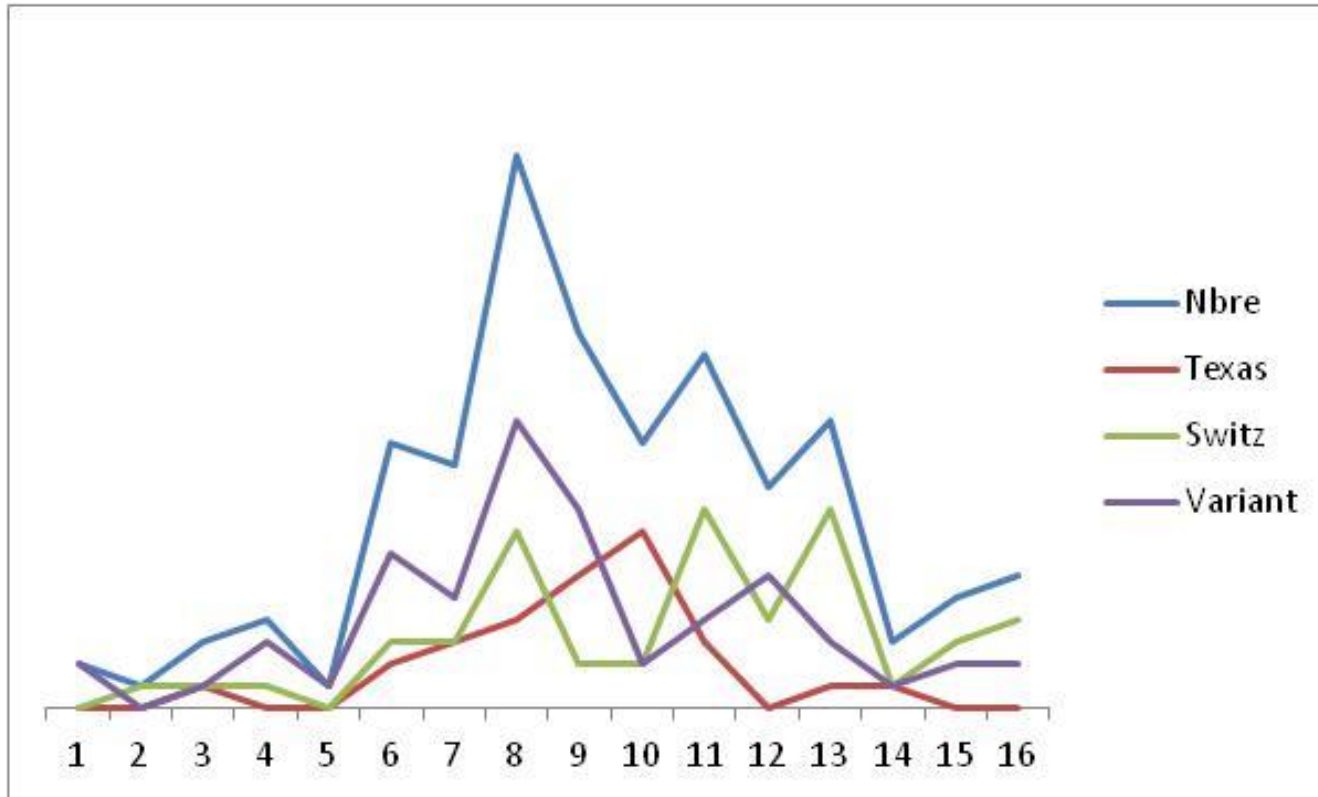
Data available at the vaccine composition meeting



Emergence de variants H3N2

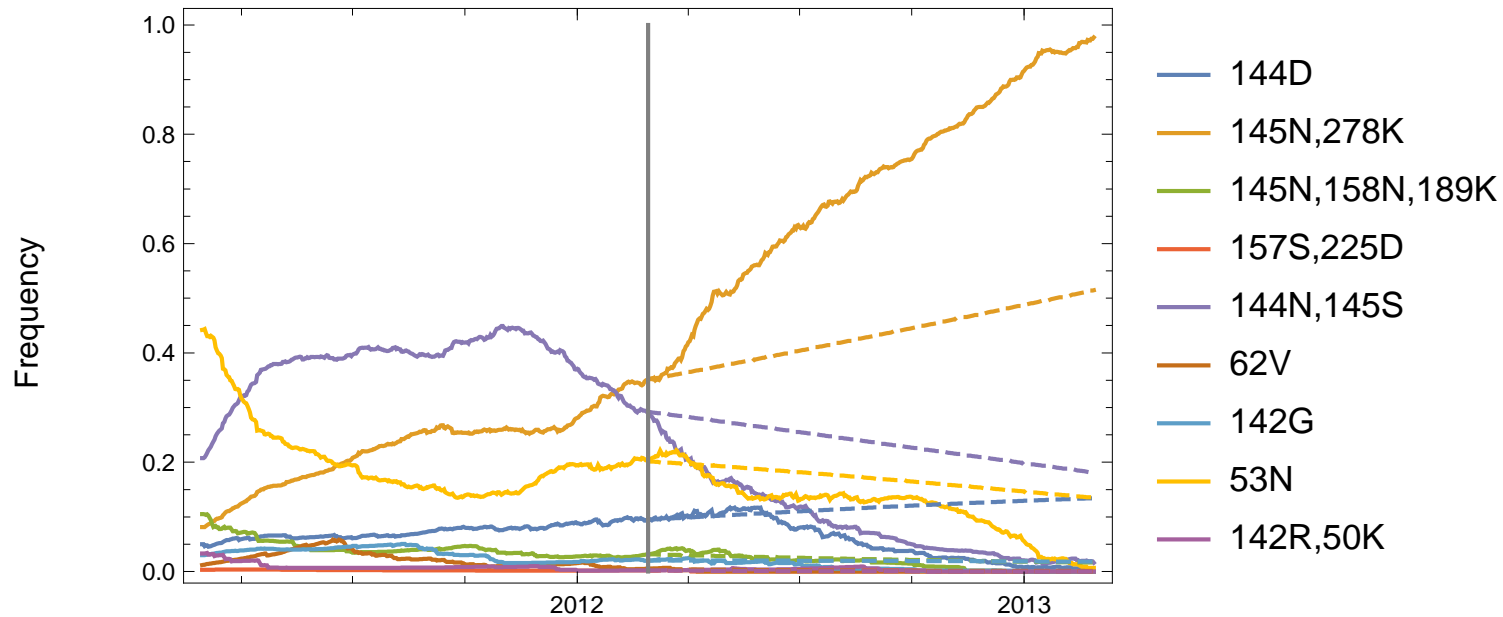


Observation d'une co-évolution des 3 lignages



Mise en oeuvre de l'outil prédiction pour le vaccine strain selection meeting

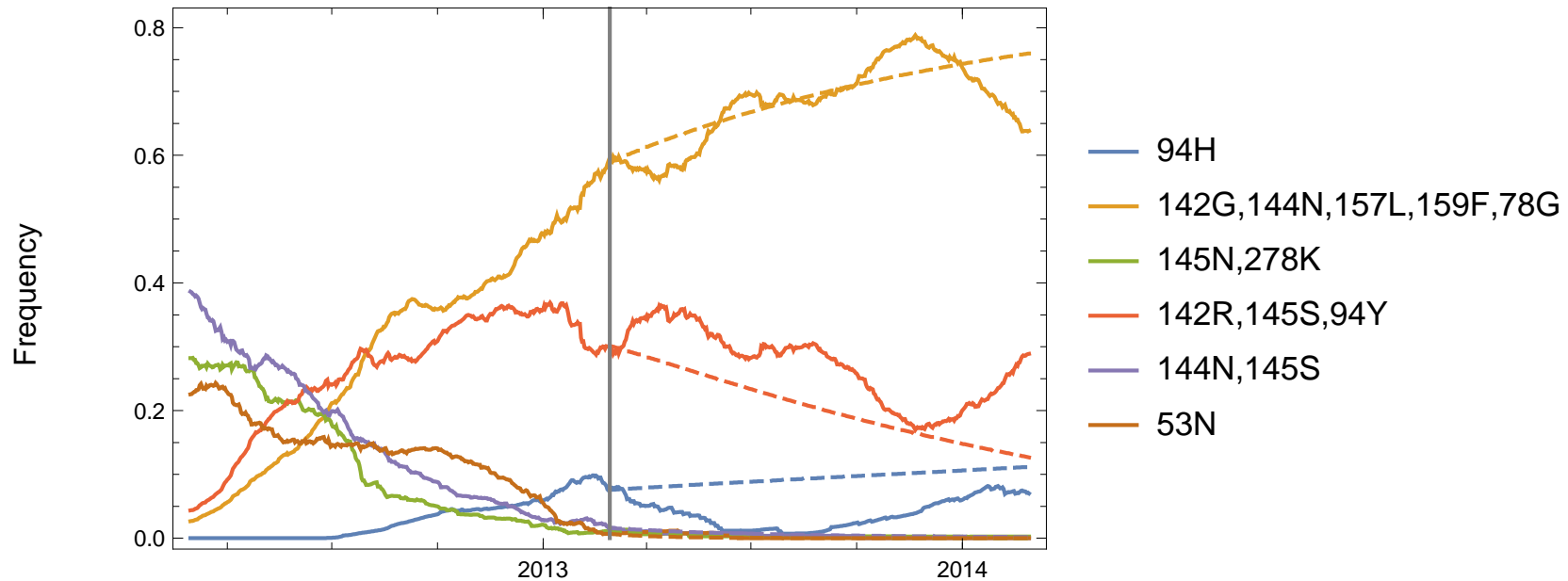
Phase 2: Clade frequency prediction



Prediction start date: February 28, 2012

Prediction period: one year

Phase 2: Clade frequency prediction

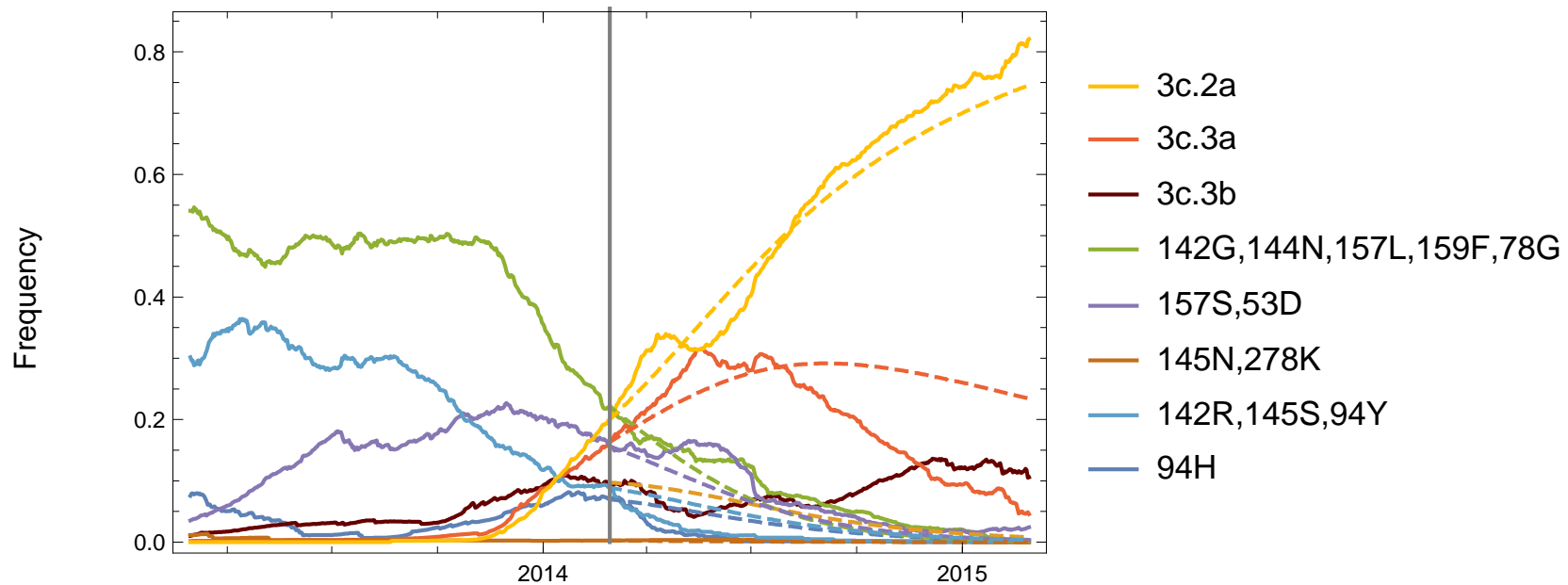


Prediction start date: February 28, 2013

Prediction period: one year

Predictions and vaccine strain selection

Phase 2: Clade frequency prediction

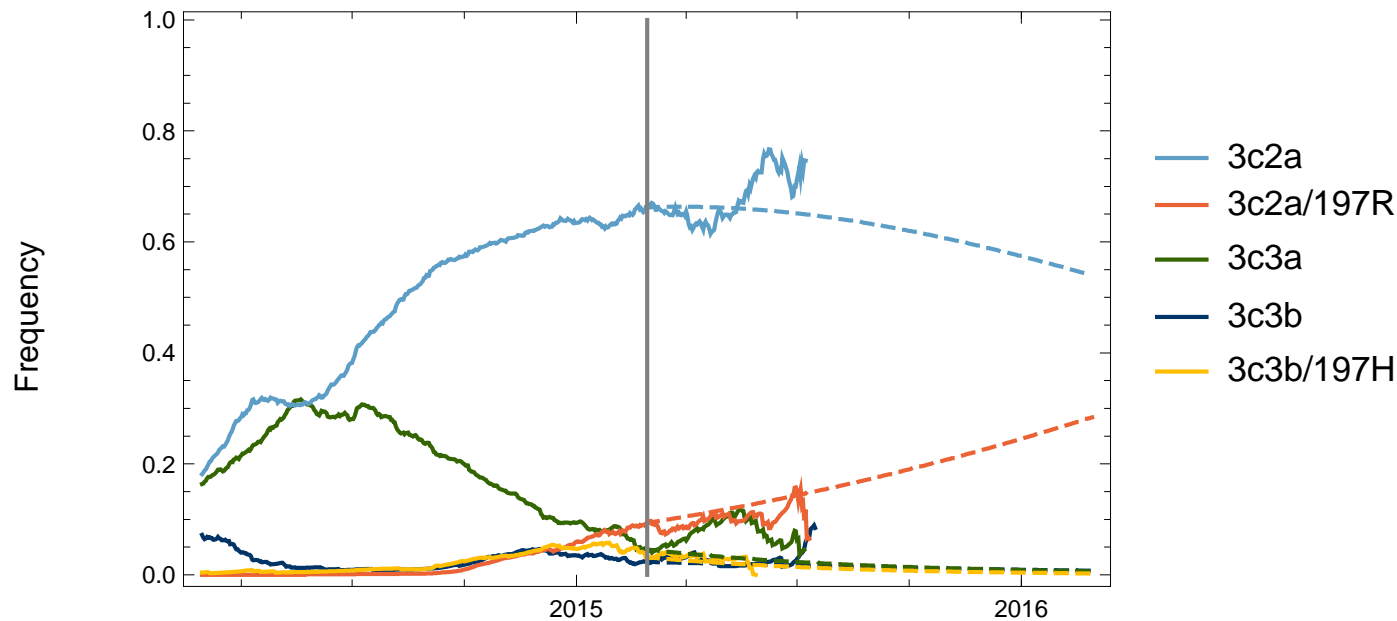


Prediction start date: February 28, 2014

Prediction period: one year

Mise en oeuvre de l'outil prédiction pour le vaccine strain selection meeting

Phase 2: Clade frequency prediction

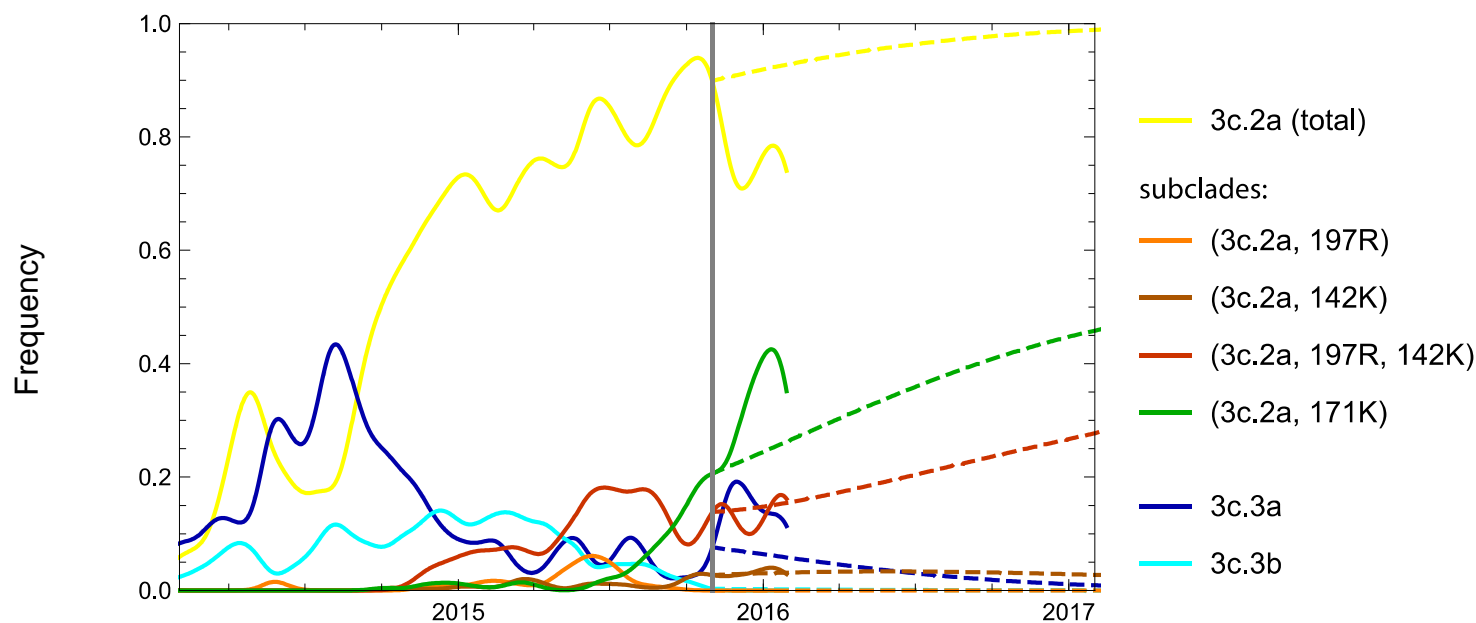


Prediction start date: February 28, 2015

Prediction period: one year

Mise en oeuvre de l'outil prédiction pour le vaccine strain selection meeting

Phase 2: Clade frequency prediction



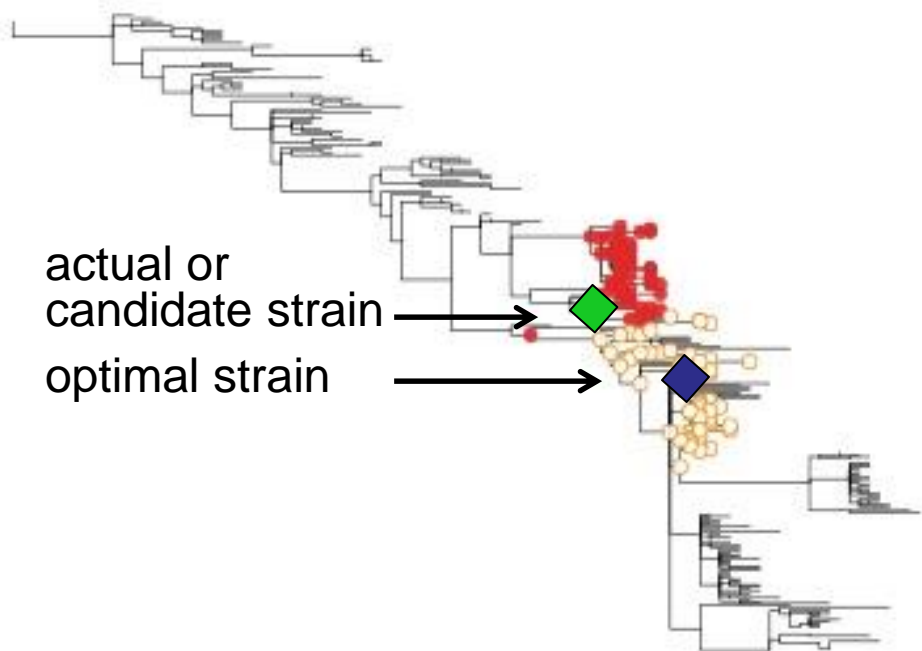
Prediction start date: February 28, 2016

Prediction period: one year

Exploitation possible de l'outil prédiction pour le VSSM

Phase 2: Prediction of vaccine efficacy

Vaccine efficacy depends on antibody-antigen binding for the (predicted) circulating strains.



[M. Luksza and M.L. 2014]

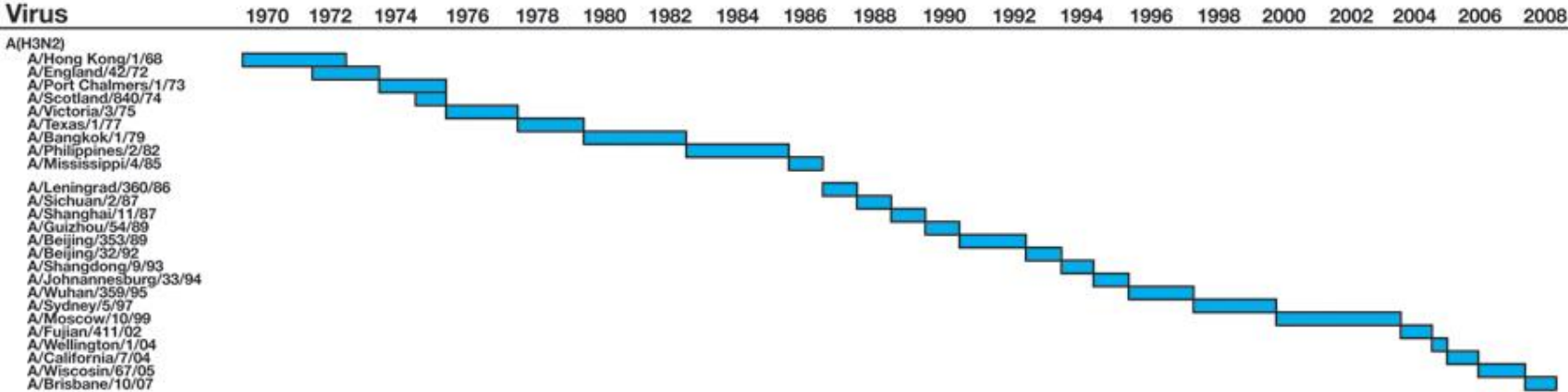
Et pour H1N1?

H1N1 situation

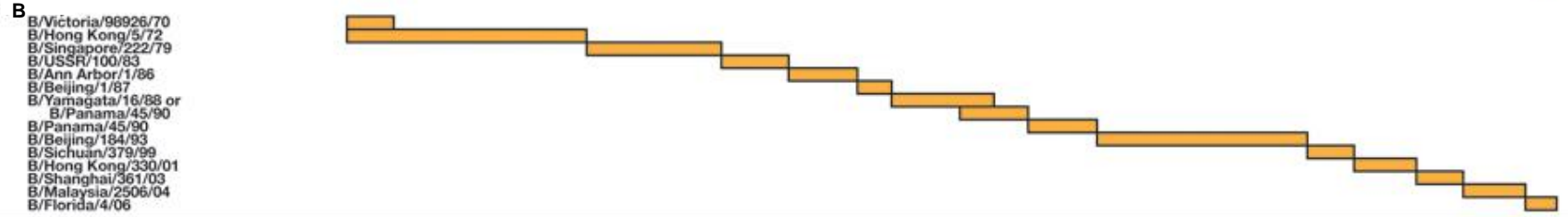
- The virus emerged in 2009 (pandemic)
- The reference strain was A/California/4/2009(H1N1)pdm09
- The second strain selected was A/California/7/2009(H1N1)pdm09
- No antigenic difference observed after 8 years (low evolution)
- Vaccine effectiveness >90% in 2009-10
- Decrease in vaccine effectiveness since with a low value of 60% this winter

- Analysis?

Change in the viruses over the years

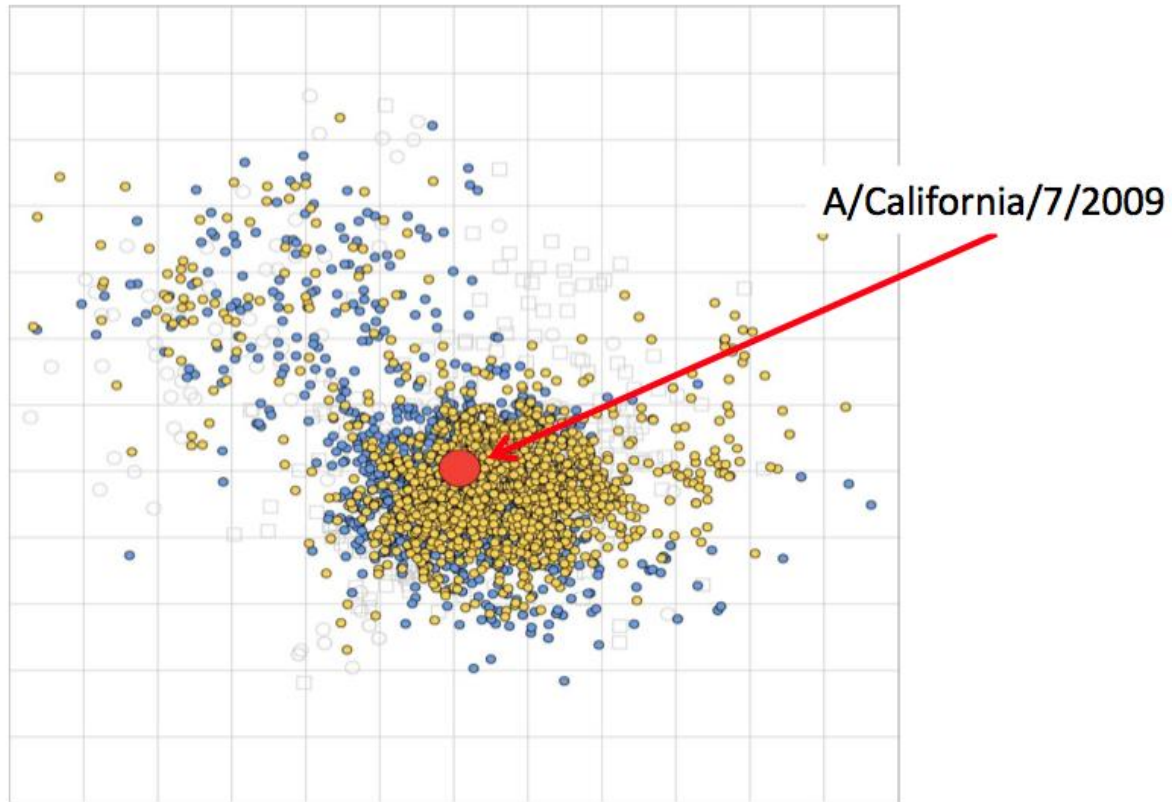


A(H1N1)
A/California/07/2009pdm



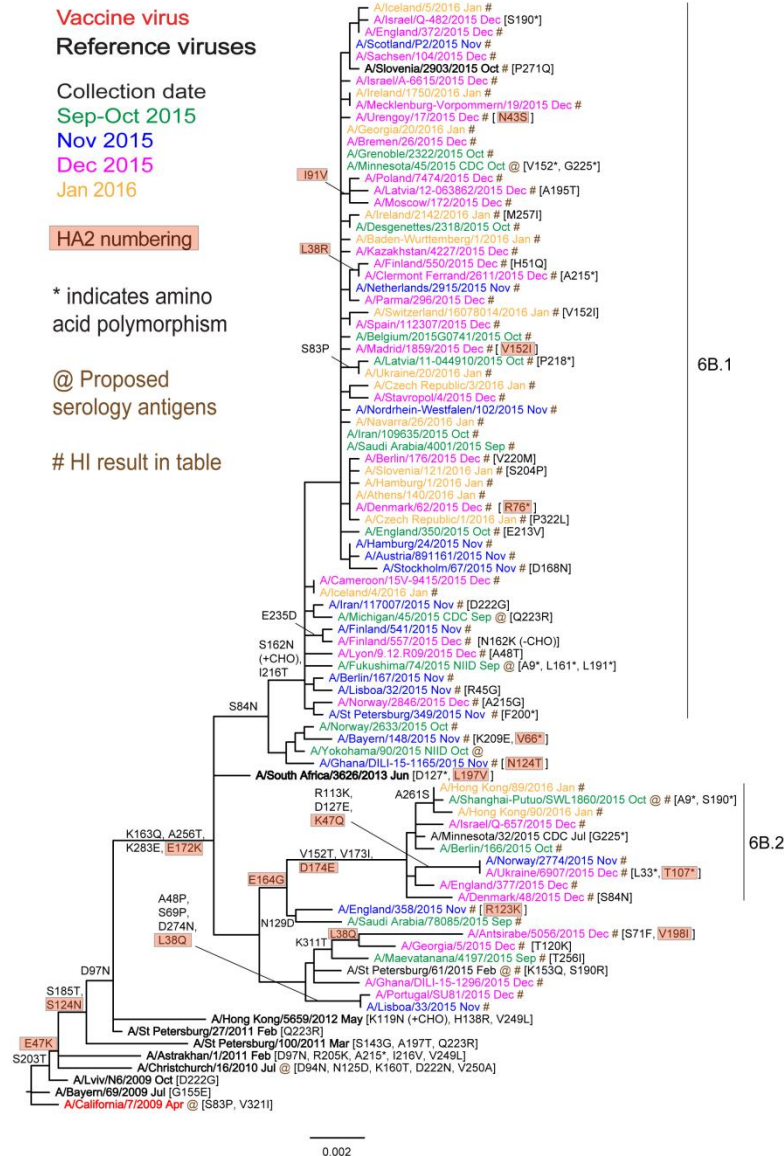
Exemple H1N1

**Antigenic cartography from Colin Russell and Derek Smith,
data from 4 CCs (NIMR, CDC, Tokyo, Japan) amalgamated**

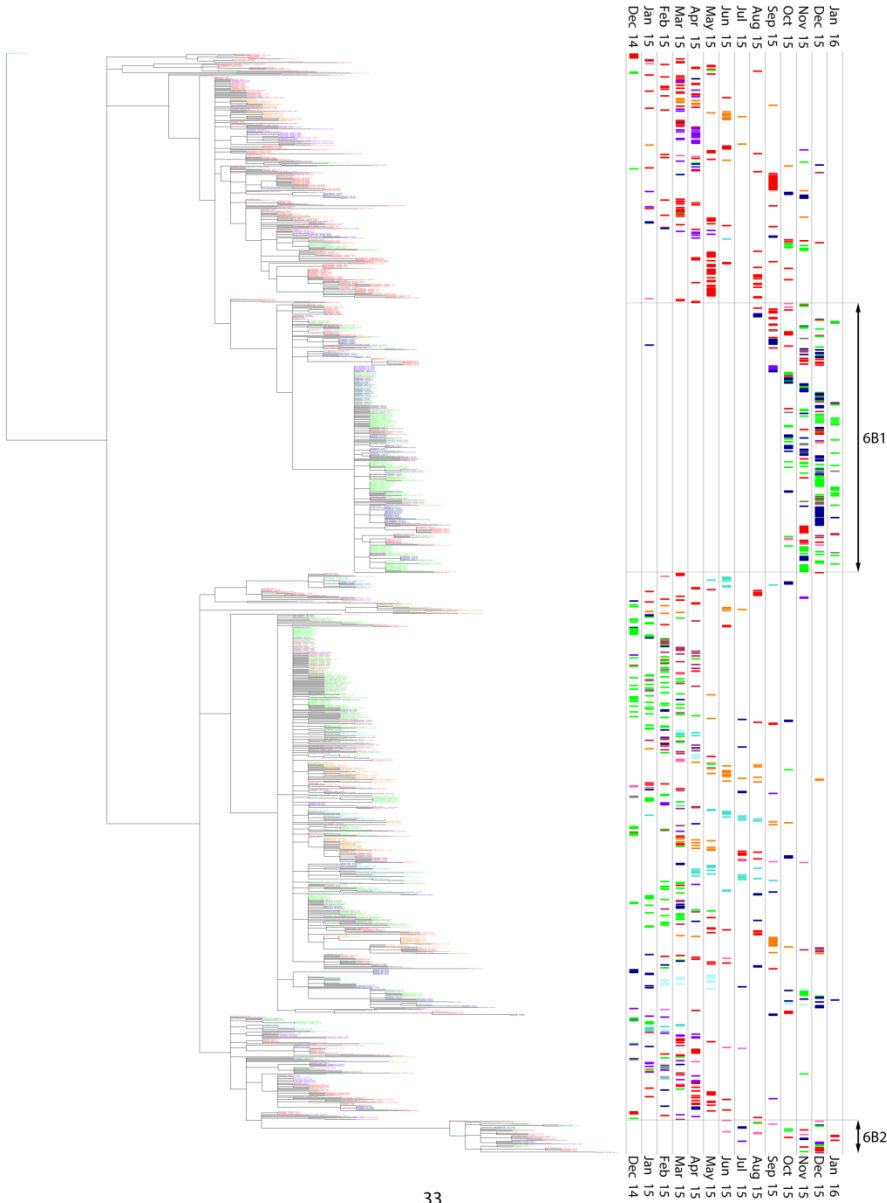


Mais...

Phylogenetic tree of HA sequences

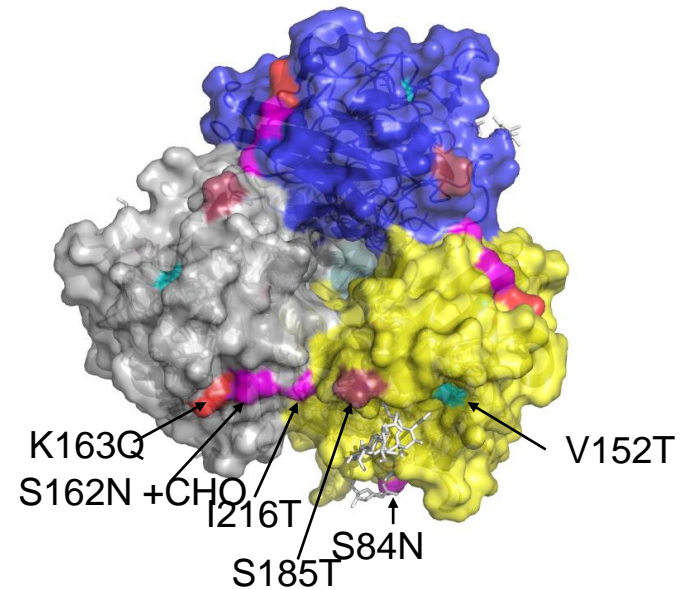
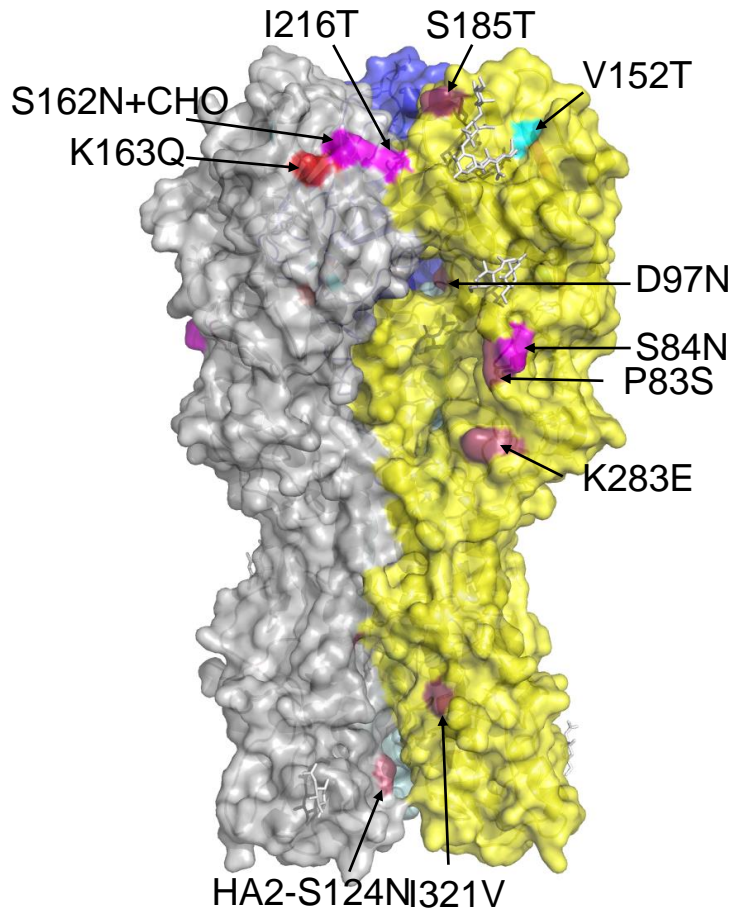


Phylogenetic Tree from Derek Smith showing the timescale of the emergence



- Code:**
- Dark Blue** = N. America,
 - Light Blue** = S. America
 - Green** = Europe
 - Orange** = Africa
 - Purple** = Middle east
 - Maroon** = Russia
 - Red** = SE Asia
 - Pink** = 'Oceania'

Position des substitutions sur H1



Changes in 6B are in **RED**

Additional Changes in 6B.1 are in **MAGENTA**

Additional Changes in 6B.2 are in **CYAN**

BROWN are other changes

Does the 6B.1 group differ antigenically from the A/California/7/2009 vaccine virus ? Analysis by HI

Antigenic analyses of influenza A(H1N1)pdm09 viruses

Viruses		Haemagglutination inhibition titre					
		Post-infection ferret antisera					
		A/Cal 7/09	A/Bayern 69/09	A/Lviv N6/09	A/Sth Afr 3626/13	A/Slov 2903/15	
		Passage history Egg	MDCK	MDCK	Egg	Egg	
	Genetic group	NIB F05/14	F09/15 ^{†1}	F14/13 ^{†1}	F3/14 ^{†1}	F02/16 ^{†1}	
		1	1	1	6B	6B.1	
REFERENCE VIRUSES							
	A/California/7/2009	1	640	320	320	1280	1280
	A/Bayern/69/2009	1	<	320	160	40	ND
	A/Lviv/N6/2009	1	80	640	640	80	ND
	A/South Africa/3626/2013	6B	640	640	640	1280	1280
	A/Slovenia/2903/2015	6B.1	2560	1280	640	2560	5120
TEST VIRUSES							
	A/Antananarivo/4551/2015	6B	1280	1280	640	2560	2560
	A/Norway/2647/2015	6B	1280	320	320	1280	2560
	A/Portugal/SU63/2015	6B	1280	640	320	1280	2560
	A/Georgia/1305/2015	6B	1280	640	640	1280	2560
	A/Portugal/SU81/2015	6B	1280	640	640	1280	2560
	A/Ghana/DILI-15-1296/2015	6B	1280	1280	640	2560	2560
	A/Georgia/1408/2015	6B	1280	1280	640	2560	5120
	A/Czech Republic/3/2016	6B.1	1280	1280	640	1280	2560
	A/Czech Republic/1/2016	6B.1	1280	640	320	1280	2560
	A/Ukraine/20/2016	6B.1	1280	640	320	1280	2560
	A/Athens/12/2016	6B.1	1280	640	320	1280	2560
	A/Ukraine/35/2016	6B.1	1280	640	320	1280	2560
	A/Hamburg/1/2016	6B.1	1280	640	320	1280	2560
	A/Baden-Wurtemberg/1/2016	6B.1	1280	640	320	1280	2560
	A/Slovenia/121/2016	6B.1	1280	640	320	1280	2560
	A/Thuringen/3/2016	6B.1	1280	640	320	1280	2560
	A/Slovenia/77/2016	6B.1	1280	640	640	1280	2560
	A/Ireland/1750/2016	6B.1	1280	640	640	1280	2560
	A/Slovenia/126/2016	6B.1	1280	640	320	1280	2560
	A/Ireland/2142/2016	6B.1	1280	640	320	1280	2560
	A/Parma/1/2016	6B.1	1280	640	640	1280	2560
	A/Athens/140/2016	6B.1	1280	640	320	1280	2560
	A/Ireland/280/2016	6B.1	1280	640	640	1280	2560
	A/Ukraine/6907/2015	6B.2	160	320	320	320	640
	A/Denmark/48/2015	6B.2	640	320	160	640	1280
	A/Norway/2774/2015	6B.2	1280	640	640	2560	2560

Does the 6B.1 group differ antigenically from A/California/7/2009? Analysis by MN

Antigenic analysis of influenza A(H1N1)pdm09 viruses - Plaque Reduction Neutralisation

Viruses	Genetic group	Neutralisation titre ¹			
		Post-infection ferret antisera			
		A/California 7/09	A/Bayern 69/09	A/South Africa 3626/13	A/Slovenia 2903/2015
		1	1	6B	6B.1
REFERENCE VIRUSES					
A/California/7/2009	1	1280	640	2560	5120
A/Bayern/69/2009	1	40	160	40	40
A/South Africa/3626/2013	6B	320	640	1280	640
A/Slovenia/2903/2015	6B.1	5120	2560	5120	10240
TEST VIRUSES					
A/Ghana/DILI-15-1281/2015	6B	5120	1280	1280	10240
A/Berlin/169/2015	6B	10240	5120	5120	10240
A/Berlin/166/2015	6B	>10240	1280	10240	10240
A/Bremen/25/2015	6B	10240	2560	10240	>10240
A/Iran/116675/2015	6B.1	1280	1280	2560	5120
A/Latvia/12-063862/2015	6B.1	2560	1280	2560	10240
A/Iran/112039/2015	6B.1	10240	2560	5120	>10240
A/England/350/2015	6B.1	5120	2560	5120	>10240
A/Berlin/168/2015	6B.1	10240	2560	5120	10240
A/Berlin/167/2015	6B.1	5120	2560	5120	10240
A/Sachsen//102/2015	6B.1	5120	5120	5120	10240
A/Nordrhein-Westfalen/103/2015	6B.1	5120	1280	5120	10240
A/England/365/2015	6B.1	5120	2560	5120	>10240
A/Bayern/147/2015	6B.1	10240	5120	5120	>10240
A/Nordrhein-Westfalen/102/2015	6B.1	5120	2560	5120	>10240
A/Iran/1337/2015	6B.1	5120	2560	5120	>10240
A/Finland/553/2015	6B.1	5120	2560	5120	>10240
A/Latvia/12-037196/2015	6B.1	5120	2560	5120	10240
A/Finland/541/2015	6B.1	5120	2560	10240	>10240
A/Netherlands/2915/2015	6B.1	5120	2560	10240	>10240
A/Latvia/12-042353/2015	6B.1	5120	2560	10240	>10240
A/Norway/2774/2015	6B.2	640	320	2560	2560

Les virus ne sont pas différents selon
les serums de furets

Y a t'il des différences avec les serums humains
et/ou
avec des monoclonaux d'origine humaine ?

HI results with post-vaccination human sera vs post-infection ferret antisera

Viruses	Genetic group	Passage history	Ferret antisera				Post-vaccination human sera					
			A/Cal 7/09 F05/14	A/Bayern 69/09 F09/15 ⁻¹ 1	A/Sth Afr 3626/13 F03/14 ⁻¹ 6B	A/Slov 2903/2015 F02/16 ⁻²	Human sera 1 AT	Human sera 2 JS	Human sera 3 NW	Human sera 4 TIV	Human sera 5 TP	Human sera 6 TRE
REFERENCE VIRUSES												
A/California/7/2009	1	NIBSC E4/E3	1280	320	640	640	160	320	80	160	320	160
A/Bayern/69/2009	1	MDCK5/MDCK1	<	320	80	<	160	160	<	80	80	80
A/Lviv/N6/2009	1	MDCK4/SIAT1/MDCK3	80	1280	80	80	640	640	80	320	320	320
A/Christchurch/16/2010	4	E1/E3	1280	1280	1280	2560	640	640	160	80	160	160
A/Astrakhan/1/2011	5	MDCK1/MDCK5	1280	640	1280	2560	320	640	80	160	320	320
A/St. Petersburg/27/2011	6	E1/E4	1280	640	1280	1280	320	640	80	160	320	320
A/St. Petersburg/100/2011	7	E1/E3	1280	1280	2560	2560	160	320	80	160	160	160
A/Hong Kong/5659/2012	6A	MDCK4/MDCK2	320	160	640	640	320	320	80	160	320	320
A/South Africa/3626/2013	6B	E1/E3	640	640	1280	1280	320	320	80	<	40	40
A/Slovenia/2903/2015		E4	1280	640	1280	2560	320	320	160	40	40	40
TEST VIRUSES												
A/Moldova/62.03/2016		MDCK1	1280	1280	1280	2560	640	640	80	<	<	40
A/Moldova/59.03/2016		MDCK1	1280	640	1280	2560	640	320	80	<	<	40
A/Moldova/45.03/2016		MDCK1	1280	640	1280	2560	640	320	80	<	<	40
A/Moldova/47.03/2016		MDCK1	1280	640	1280	2560	640	640	80	<	<	40
A/Moldova/48.03/2016		MDCK1	640	320	1280	2560						
B/Istanbul/201/2015		MDCK1/MDCK1	1280	640	1280	2560	80	80	<	<	<	<
A/Oman/6268/2015		SIAT2/MDCK1	1280	640	1280	2560	640	640	80	<	<	40
A/Oman/6337/2015		SIAT2/MDCK1	1280	640	1280	2560	80	40	80	<	<	80
A/Oman/6338/2015		SIAT2/MDCK1	1280	640	1280	2560						
A/Oman/6329/2015		SIAT2/MDCK1	1280	640	1280	2560	640	640	80	<	<	40
A/Greece/65/2016		MDCK1	1280	640	1280	2560	640	640	80	<	<	40

Human Monoclonal antibody recognition of test viruses

Viruses		Haemagglutination inhibition titre										
		Post-infection ferret antisera				Human monoclonal antibodies ²						
		A/Cal	A/Bayern	A/Sth Afr	A/Slov	1	2	3	4	5	6	7
		7/09 Egg	69/09 MDCK	3626/13 Egg	2903/15 Egg	T2-6A ²	T2-7D ²	T2-8A ²	T2-11C ²	T2-5D ²	T1-9B ²	2-12C ²
Ferret number	NIB F05/14 ⁻¹	F09/15 ⁻¹	F3/14 ⁻¹	F02/16 ⁻¹								
Genetic group	1	1	6B									
REFERENCE VIRUSES												
A/California/7/2009	1	1280	640	1280	2560	2560	10240	640	640	320	320	2560
A/Bayern/69/2009	1	<	160	40	<	<	<	<	<	<	<	<
A/Christchurch/16/2010	4	1280	1280	1280	2560	320	2560	2560	40	80	40	5120
A/Astrakhan/1/2011	5	1280	640	1280	2560	2560	5120	5120	320	320	160	2560
A/St. Petersburg/27/2011	6	1280	1280	1280	2560	5120	20480	20480	640	320	320	5120
A/St. Petersburg/100/2011	7	2560	1280	1280	2560	5120	20480	20480	640	640	320	5120
A/Hong Kong/5659/2012	6A	320	160	320	640	1280	5120	5120	40	<	<	1280
A/South Africa/3626/2013	6B	1280	640	1280	1280	<	<	<	<	<	<	2560
TEST VIRUSES												
A/Slovenia/2903/2015	6B.1	1280	640	1280	2560	<	<	<	<	<	<	2560
A/Slovenia/2903/2015	6B.1	2560	640	1280	2560	<	<	<	<	<	<	2560
A/Scotland/P2/2015	6B.1	1280	640	1280	2560	<	<	<	<	<	<	2560
A/Lisboa/30/2015		640	320	640	2560	<	<	<	<	<	<	2560
A/Iran/116675/2015		1280	640	1280	2560	<	<	<	<	<	<	1280
A/Iran/109635/2015		1280	640	1280	2560	<	<	<	<	<	<	2560
A/St. Petersburg/349/2015		1280	640	1280	2560	<	<	<	<	<	<	2560
A/Finland/546/2015		640	320	1280	2560	<	<	<	<	<	<	1280
A/England/357/2015		1280	640	640	2560	<	<	<	<	<	<	2560
A/Antsirabe/4186/2015		1280	640	1280	2560	<	<	<	40	<	<	2560
A/Maevatanana/4189/2015		1280	640	1280	2560	<	<	<	<	<	<	1280
A/Mecklenburg-Vorpommern/19/2015		640	320	640	1280	<	<	<	<	<	<	1280
A/Bayern/148/2015		640	320	640	1280	<	<	<	<	<	<	1280
A/Austria/891161/2015		640	320	640	1280	<	<	<	40	<	<	1280

Constat

Deux sous clades de H1N1 ont émergé rapidement
Cette émergence s'est faite dans un contexte général de réduction de
l'efficacité vaccinale
H1N1pdm09 est significativement associé à des formes sévères

Les virus appartenant à ces sous clades (6b1 et 6b2) ne sont pas distincts
de la souche prototype vaccinale (selon les sérums de furets)

Ces virus apparaissent comme différents (ou montrant des différences)
lorsqu'on utilise des monoclonaux humains

Les sérums humains post vaccinaux donnent des résultats de
caractérisation difficile à interpréter.

Donc :

Évolution vers la souche Michigan (clade 6b1)

Sans données objective de variation antigénique majeure

Mais en s'appuyant sur les données de fitness générées par M Laessig

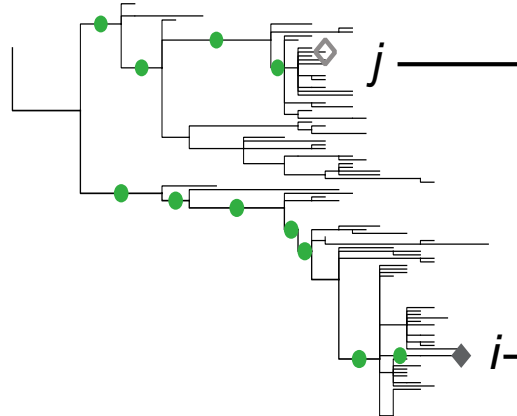
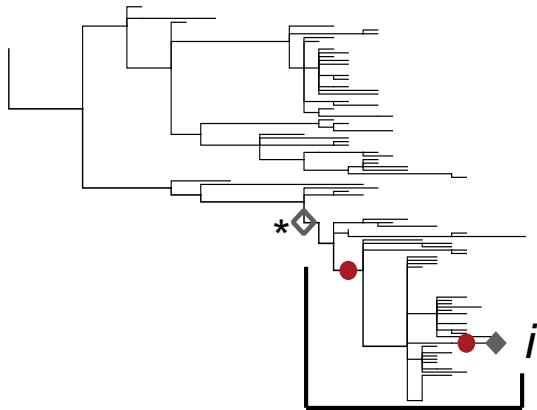
Données utilisées pour les études de fitness

Les données combinées phylogénétiques/antigéniques des HA

non-epitope mutations:
decrease protein stability
→ *decrease fitness*

Major epitope mutations:
decrease binding affinity
→ *increase fitness*

Minor epitope mutations:
decrease binding affinity
→ *Impact on fitness?*



[adapted from M. Luksza and M.L. 2014]

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