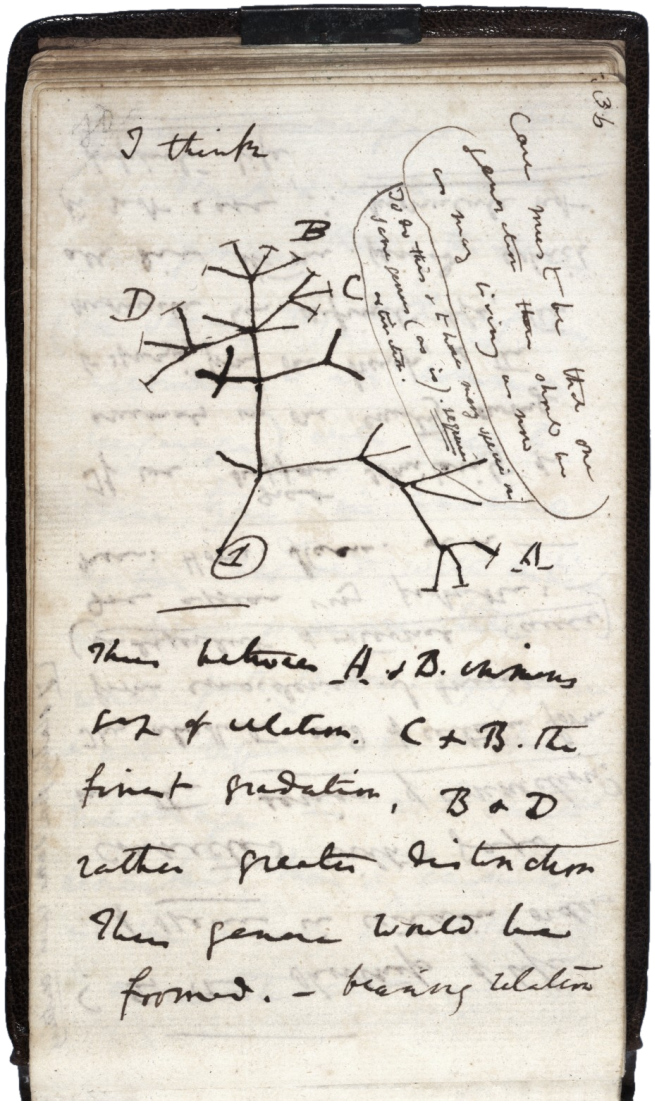
Evolution

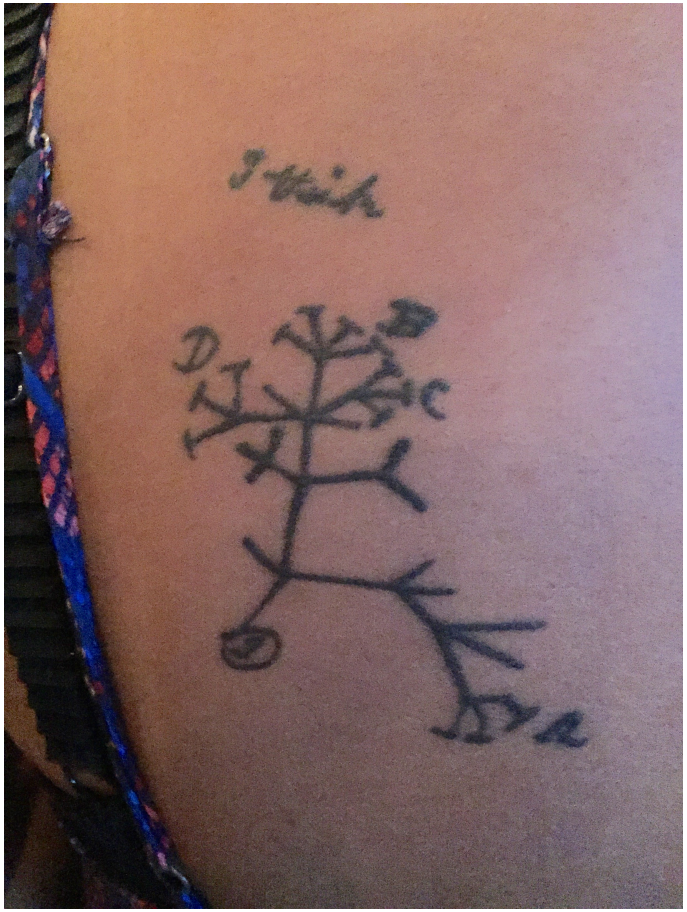
Session 21

Virology Live

Fall 2021

Around here, it takes all the running you can do just to stay in the same place.
Lewis Carroll
Alice in Wonderland





http://commons.wikimedia.org/wiki/File:Darwin_Tree_1837.png

Adaptation

Host protein adapted
to environment

Change in environment/
selective regime

Host protein maladapted
to environment

Adaptive evolution

Host protein adapted
to "new" environment

Purifying selection

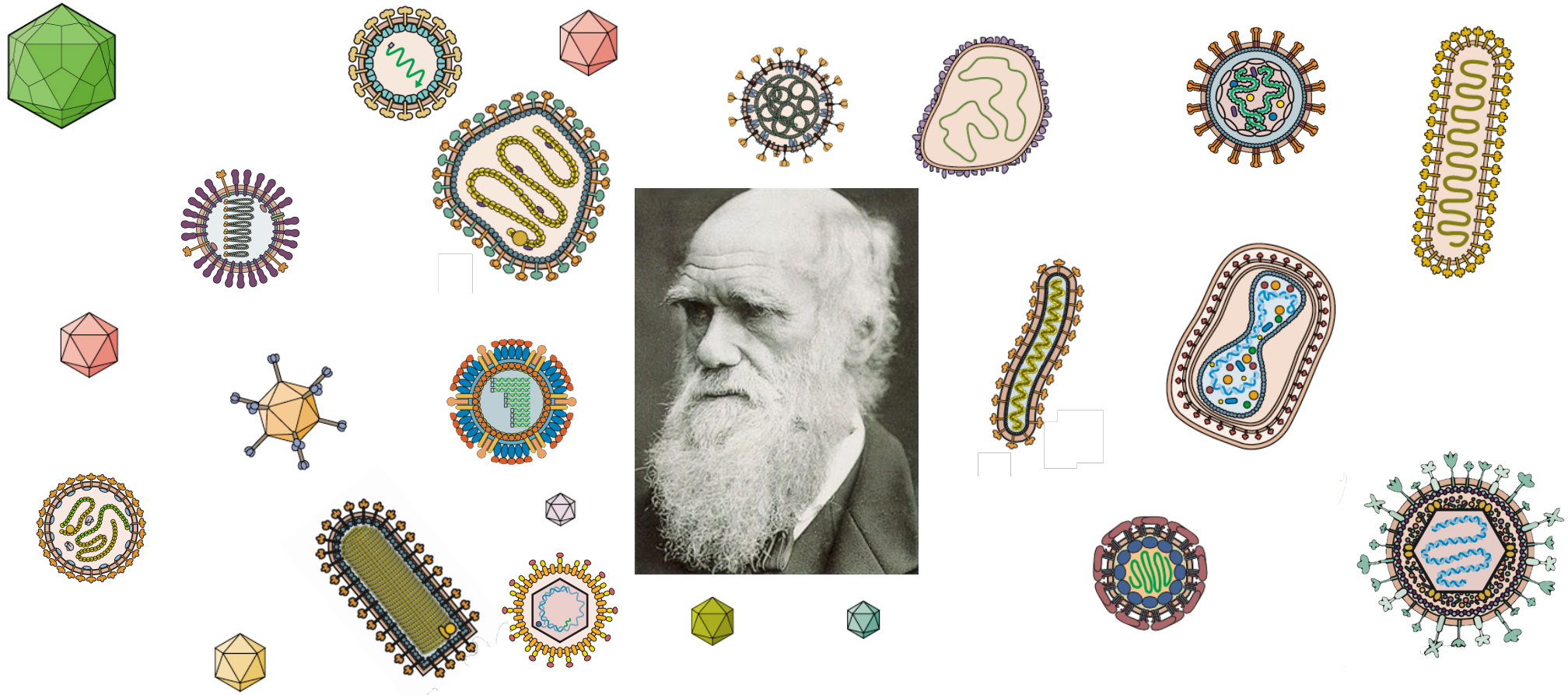
(removal of deleterious alleles)

Host protein maintained
in "new" environment

(positive selection)

Annu. Rev. Genet. 2012. 46:677–700

Darwin would have loved viruses!



The best exemplars of evolution by natural selection, and for RNA viruses, evolution is so rapid it can be followed in real time

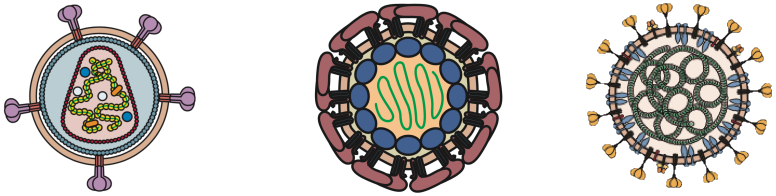
Viral evolution: The constant change of a viral population in the face of selection pressures

- Where did viruses come from?
- Where are viruses going?

Modern virology has provided a window on the mechanisms of evolution

- As host populations grow and adapt, virus populations are selected that can infect them
  - *New viral populations emerge every day*
- It also works the other way
  - *Viral populations can be significant selective forces in the evolution of host populations*
- If a host population cannot adapt to a lethal virus infection, the population may be exterminated

The public is constantly confronted with the reality of viral evolution (even if they don't believe in evolution)

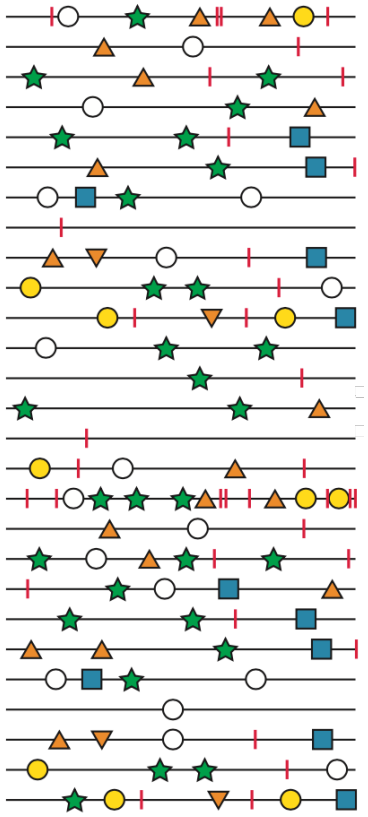


- New viral diseases: AIDS, West Nile virus disease in the US, hepatitis C, Ebolavirus disease, Zika virus disease, COVID-19
- Regular bouts every year with influenza and common cold viruses
- Drug resistant HIV-1

Simple fact: virus evolution is faster than many can comprehend

Four main drivers of virus evolution

- Large numbers of progeny
- Large numbers of mutants
- Quasi-species effects
- Selection



Virus-infected cells produce large numbers of progeny

Virus in plasma	HBV	HIV
Half-life	24 h	6 h
Daily turnover	50%	90%
Total production in blood	$>10^{11}$	$>10^{9}$

The interface of host defense and virus replication is fertile ground for selection and evolution

Replicating viruses produce large numbers of mutant genomes

- Evolution is possible only when mutations occur in a population
- Mutations are produced during copying of any nucleic acid molecule

Viral genomes are always mutating!

HEALTH EBOLA

The Ebola Virus Is Mutating, Say Scientists

TIME

Kevin McSpadden @KevinMcspadden | Jan. 29, 2015

The outbreak has so far claimed 8,795 lives across the affected West African region

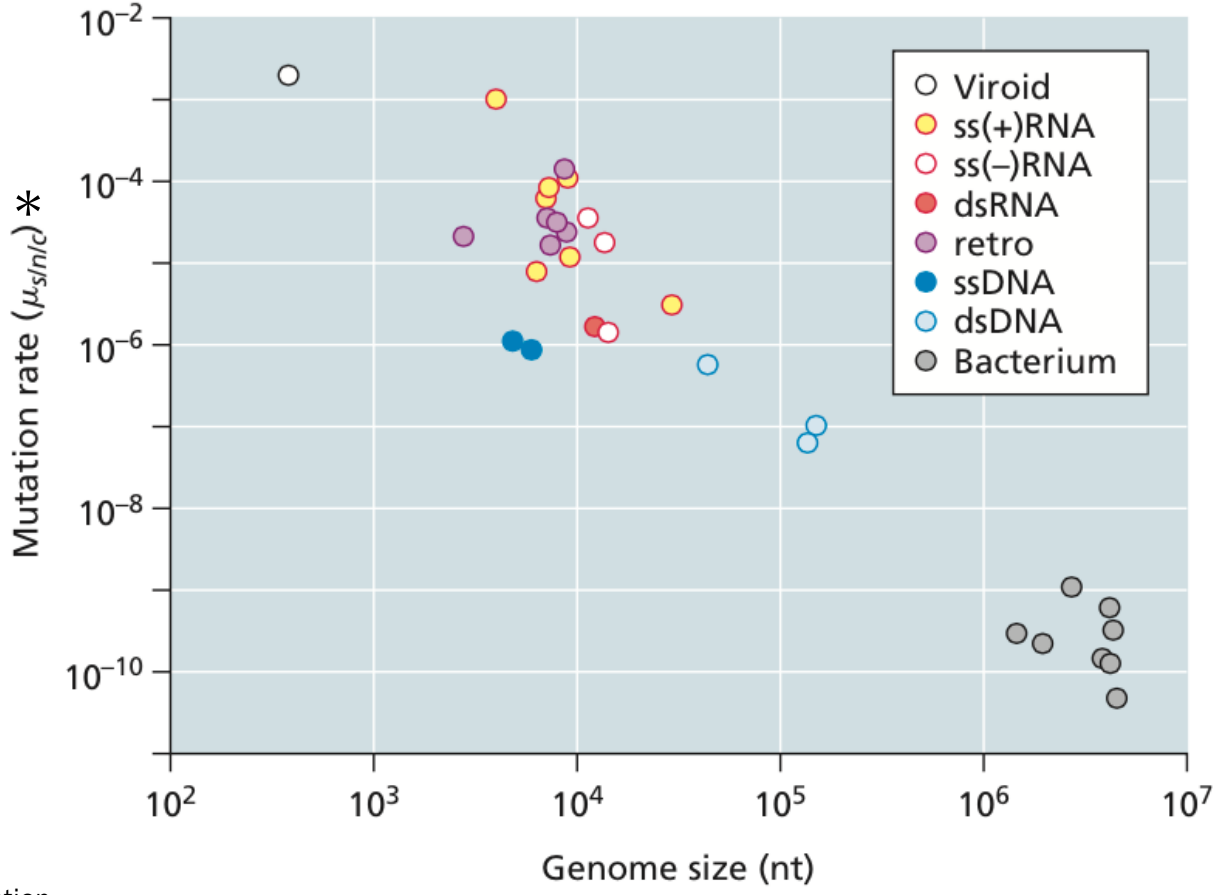
Scientists at a French research institute say the Ebola virus has mutated and they are studying whether it may have become more contagious.

Researchers at the Institut Pasteur are analyzing hundreds of blood samples from Guinean Ebola patients in an effort to determine if the new variation poses a higher risk of transmission, according to the BBC.

Youssouf Bah—AP

A health care worker, right, takes the temperatures of school children for signs of the Ebola virus before they enter their school in the city of Conakry, Guinea, Monday, Jan. 19, 2015

The Coronavirus Is Mutating. What Does That Mean for Us?



*substitutions/nucleotide/generation

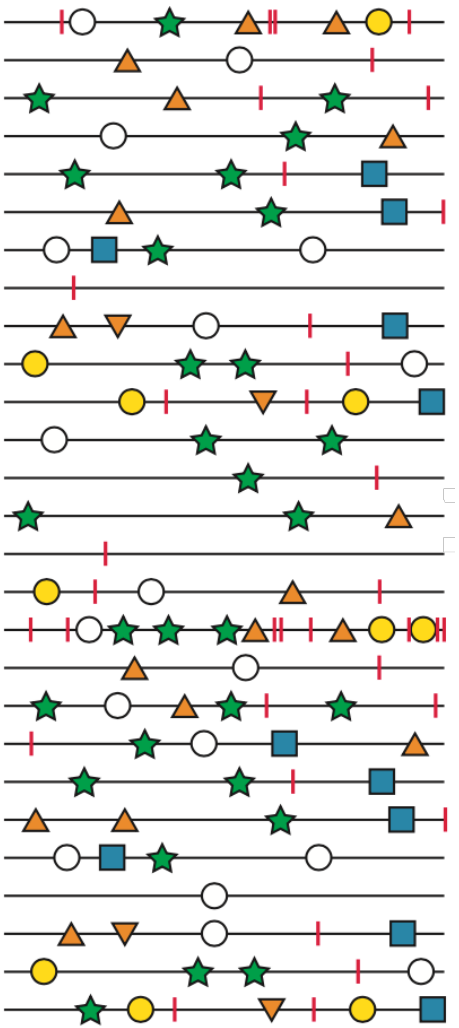
The quasispecies concept

- Analysis of an RNA bacteriophage population (Qβ):

  "A Qβ phage population is in a dynamic equilibrium with viral mutants arising at a high rate on the one hand, and being strongly selected against on the other. The genome of Qβ cannot be described as a defined unique structure, but rather as a weighted average of a large number of different individual sequences." E. Domingo, D. Sabo, T. Taniguchi, C. Weissmann. 1978. Nucleotide sequence heterogenity of an RNA phage population. Cell 13:735-744.

- This discovery was far ahead of its time, not appreciated by most virologists
- Virus populations exist as dynamic distributions of nonidentical but related replicons, called *quasispecies*
- Some prefer the term *mutant swarm* or *cloud*

Viral quasispecies



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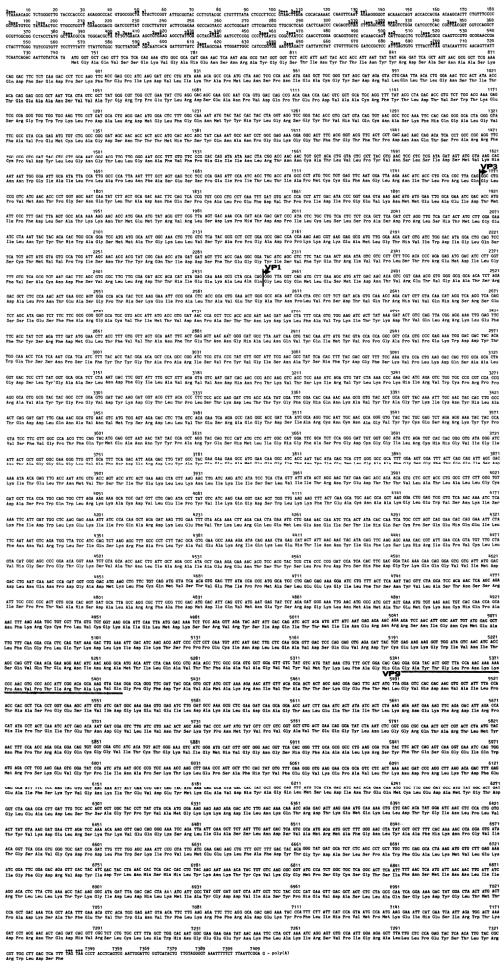
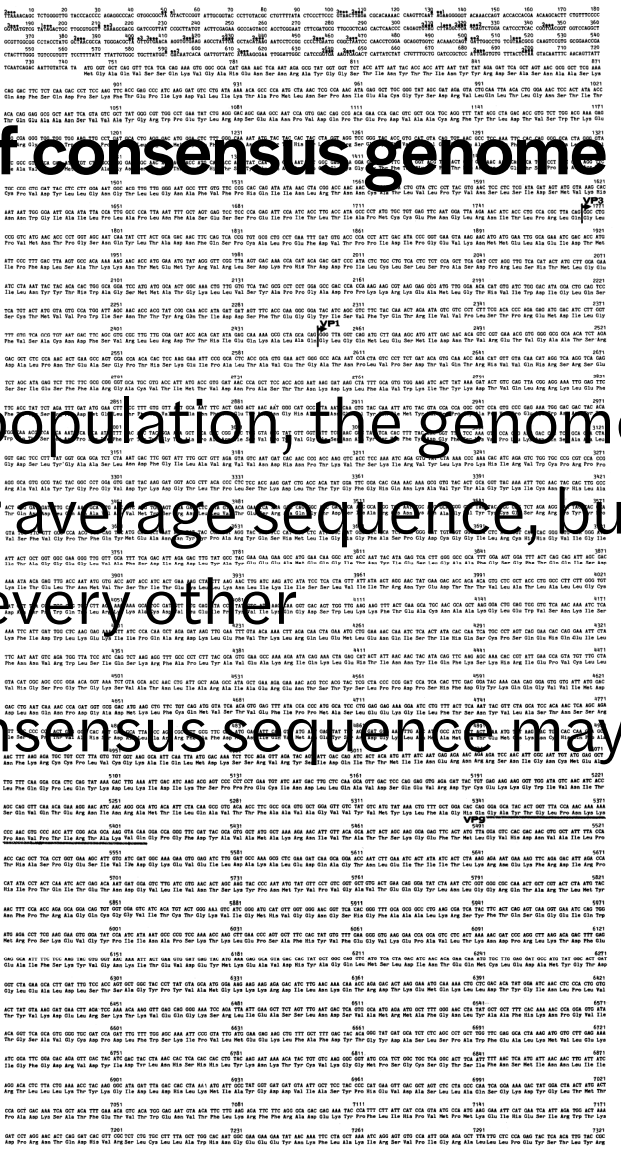


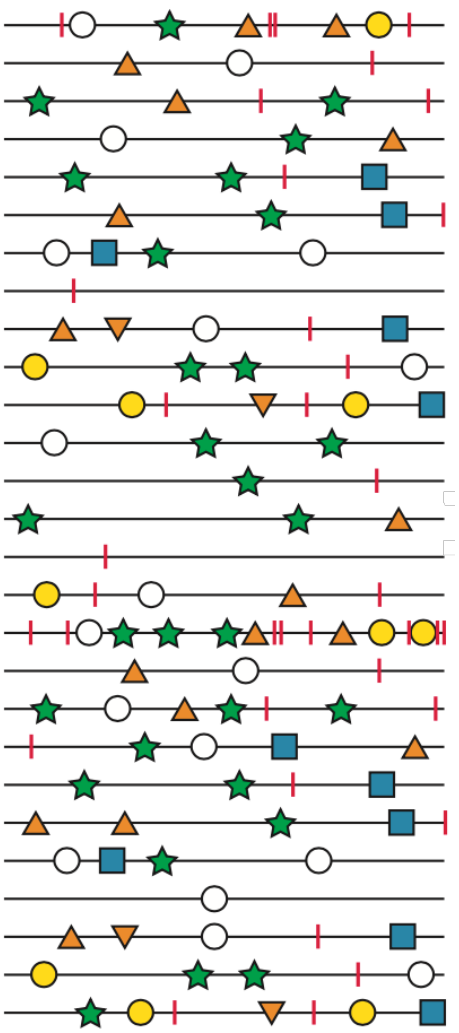
The myth of consensus genome sequences



- For a given RNA virus population, the genome sequences cluster around a consensus or average sequence, but virtually every genome can be different from every other
- A genome with the consensus sequence may not exist in the population

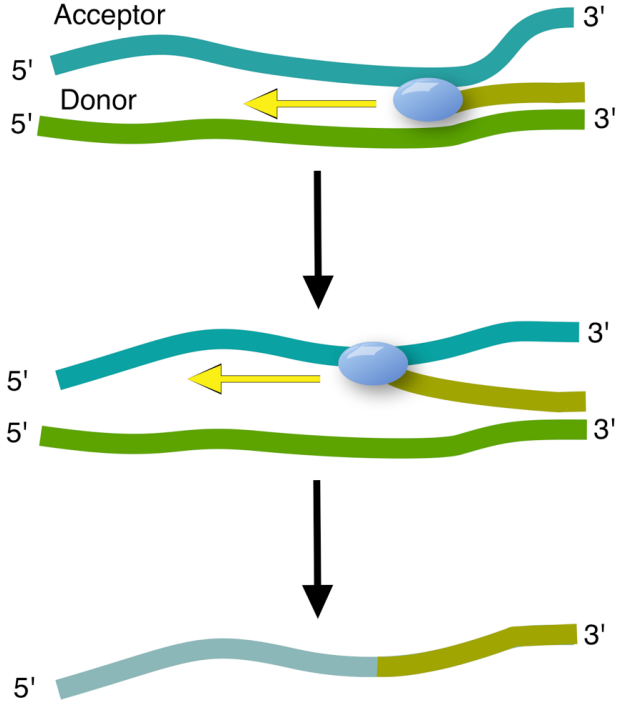
Quasispecies effects

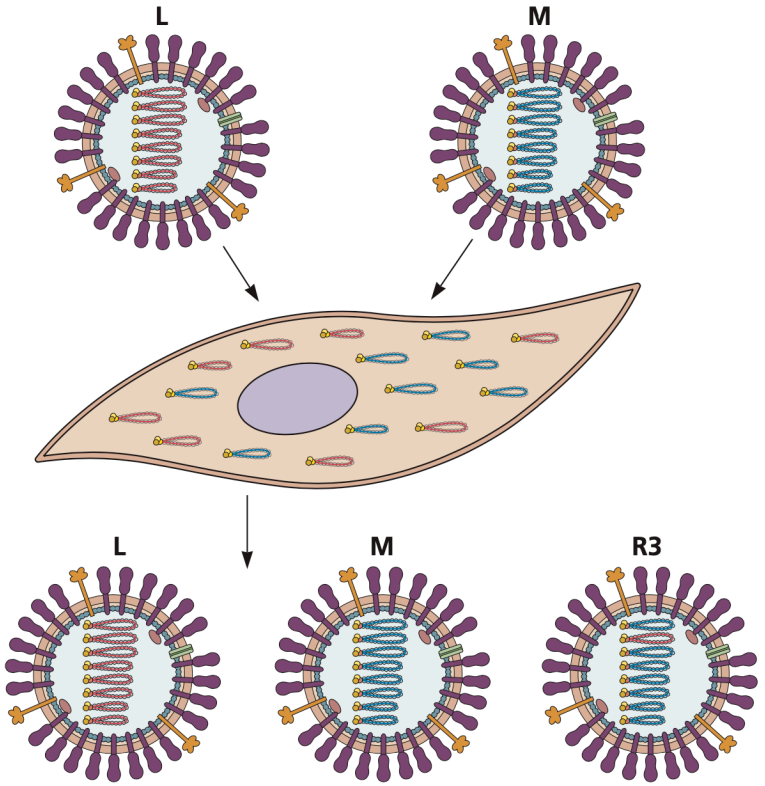
- Viral infections are initiated by a population of genomes, not a single virus genome
- The large number of progeny produced are products of selective forces inside the host
- The survivors that can re-infect a new host reflect the selective forces outside the host



Quasispecies

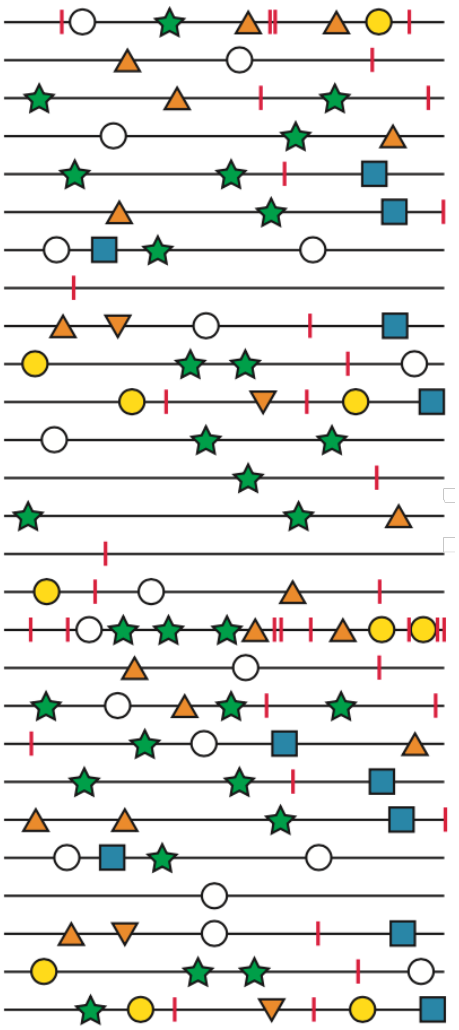
Variation further generated by recombination and reassortment



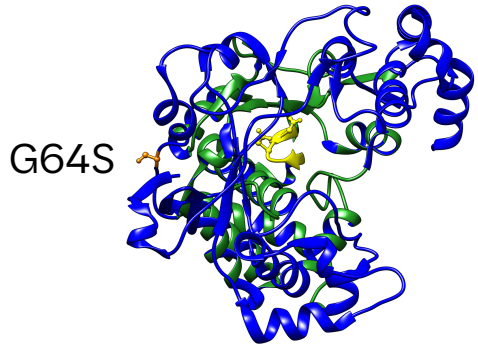


Selection

- *Survival of the fittest*: A rare genome with a particular mutation may survive a selection event, and this mutation will be found in all progeny genomes
- *Survival of the survivors*: However, the linked, but unselected mutations, get a free ride
- Consequently, the product of selection after replication is a new, diverse population that shares the selected and closely linked mutations



Diversity is selected



- Mutations in viral polymerases that reduce the frequency of incorporation errors
  - Do **not** have a selective advantage when wild type and anti-mutators are propagated together
  - Lower rates are neither advantageous nor selected in nature
  - Mutants are often less pathogenic
- High mutation rates are selected during virus evolution: mutation is good for viral populations

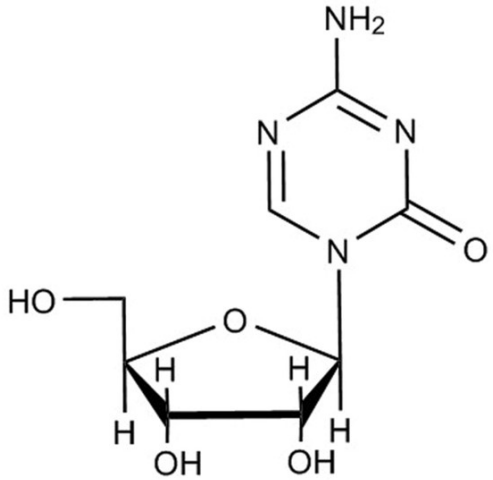
http://www.virology.ws/2009/05/15/increased-fidelity-reduces-viral-fitness/

Error threshold

- Mutation is a powerful advantage, but selection and survival balances genetic fidelity and mutation rate
- This limit is called the **error threshold**
  - Exceed it: loss of infectivity
  - Below it: cannot produce enough mutations to survive selection
- RNA viruses: evolve close to their error threshold
- DNA viruses: evolve far below their error threshold

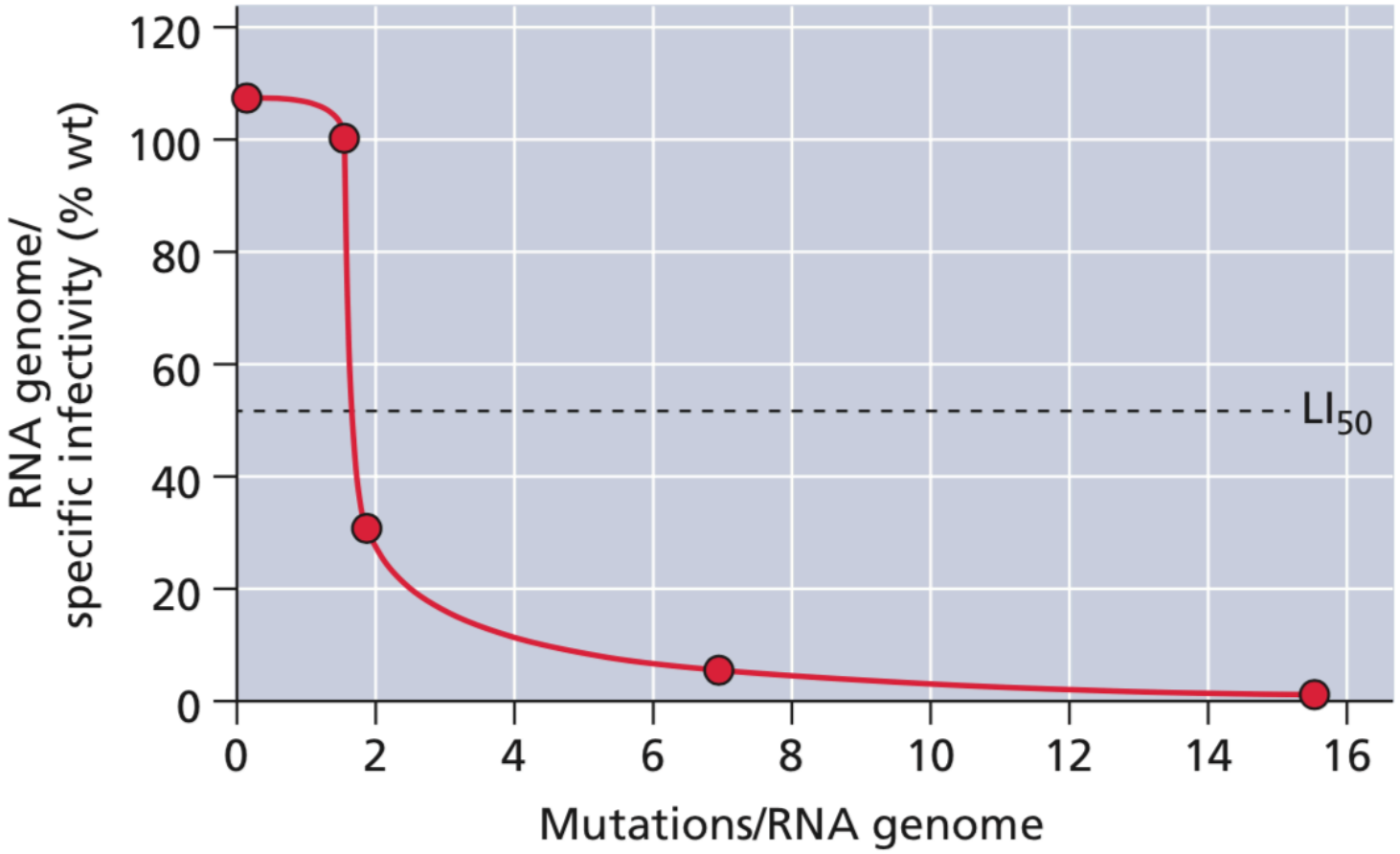
Error threshold

- Expose a cell culture infected with a DNA virus to a base analog such as 5-azacytidine
- 5-azacytidine is incorporated as a C, but templates as a T (G to A transitions)
- Mutation rate among viral progeny increases several orders of magnitude
- When a similar experiment is done with an RNA virus, the error frequency per genome increases only two- to threefold at best - cannot make any more mutations



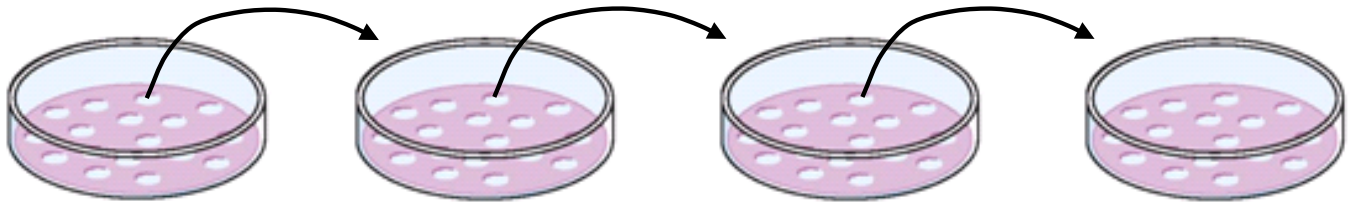
Error threshold

Antiviral *ribavirin* and poliovirus



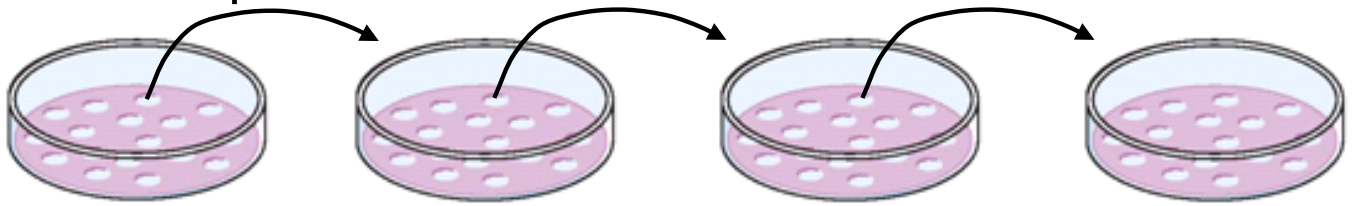
Importance of Quasispecies: Genetic bottlenecks

- Extreme selective pressures on small populations that result in loss of diversity, accumulation of non-selected mutations, or both
- A single RNA virus plaque is picked and expanded
- Next, a single plaque is picked from the expanded stock
- The process is repeated over and over



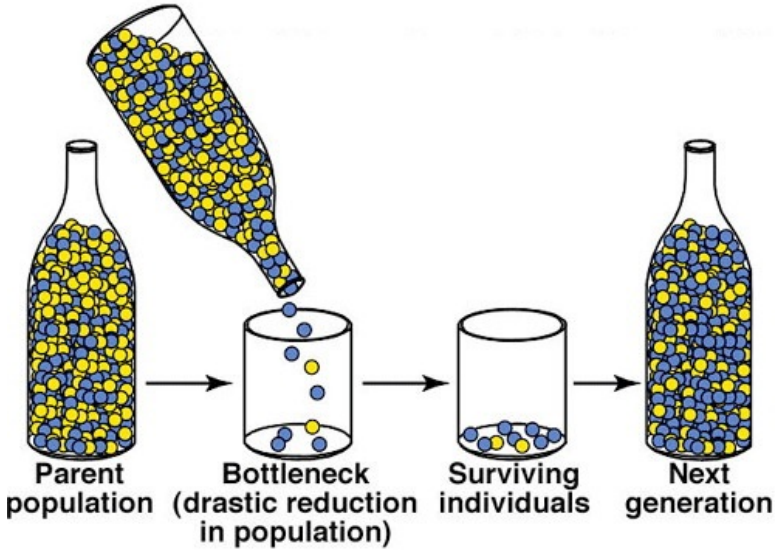
Genetic bottlenecks

- After about 20-30 cycles of single-plaque amplification, many virus populations are barely able to grow
- They are markedly less fit than the original population
- The environment is constant, and the only apparent selection is that imposed by the ability of the population of viruses from a single plaque to replicate
- Why does fitness plummet?



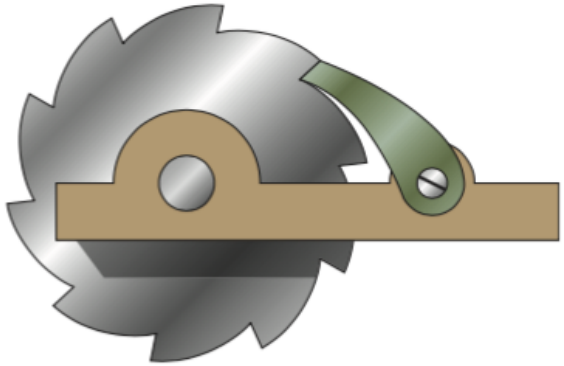
Genetic bottlenecks

- The bottleneck arises by restricting further viral replication to the progeny found in a single plaque
  - A few thousand progeny viruses derived from a single founder virus



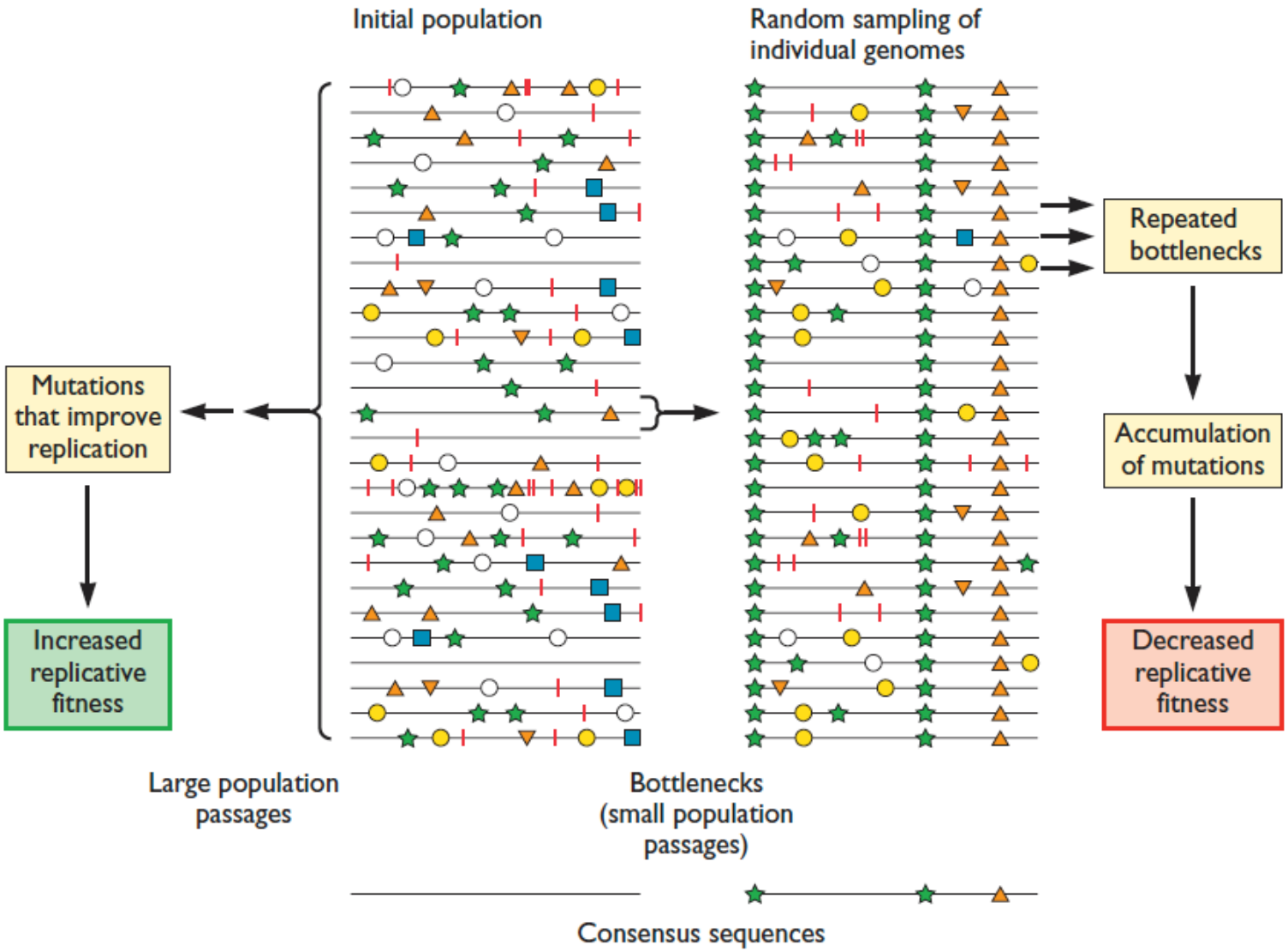
Genetic bottlenecks

- Another way to look at this problem: **Muller's ratchet**: Small, asexual populations accumulate deleterious mutations
- Replicating RNA viruses are close to error threshold
- By restricting population growth to serial single founders (the bottleneck) under otherwise nonselective conditions, so many mutations accumulate (exceed the threshold) that fitness decreases



The ratchet metaphor: each of the new mutations works like a ratchet, allowing the gear to move forward, but not backward

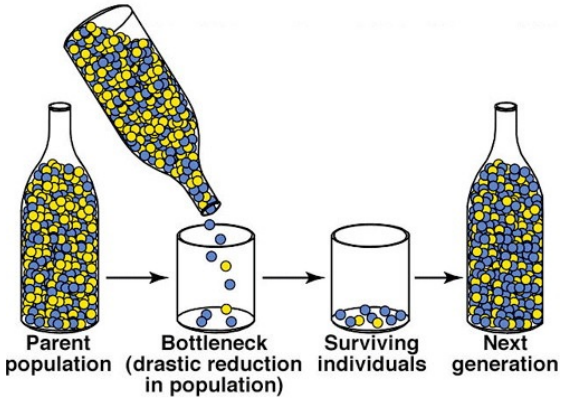
Each round of error-prone replication works like a ratchet, “clicking” relentlessly as mutations accumulate at every replication cycle



Fitness decline compared to initial virus clone after passage through a bottleneck

Virus	# of bottleneck passages	% Decrease in fitness
Bacteriophage ϕ6	40	22
Vesicular stomatitis virus	20	18
Foot-and-mouth disease virus	30	60
HIV	15	94
Bacteriophage MS2	20	17

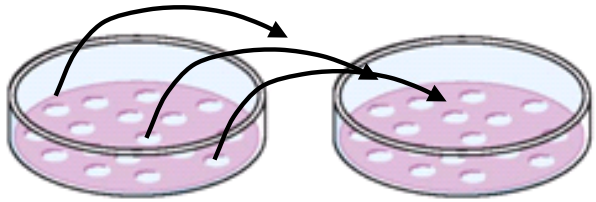
Bottlenecks in the real world?



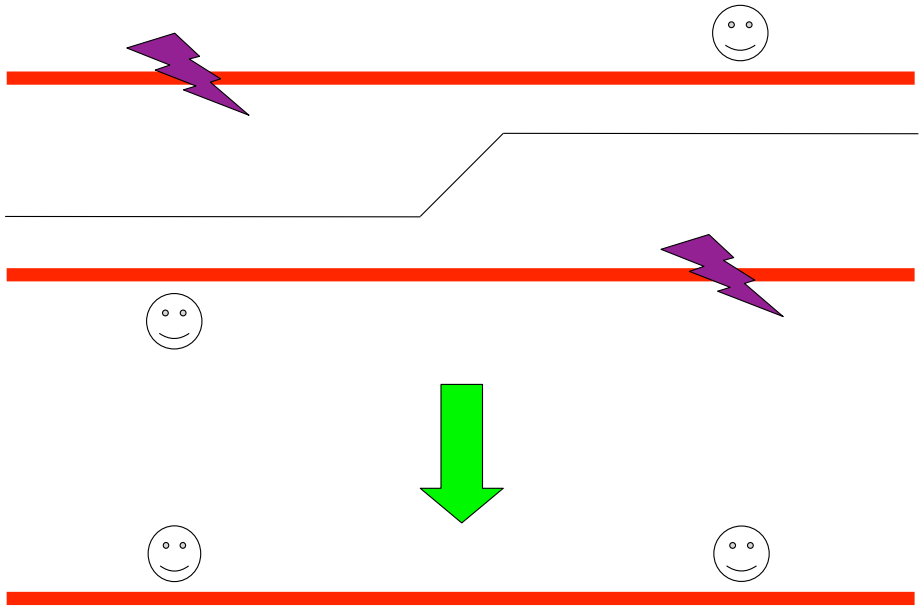
- Infection by a limited virus population and subsequent amplification are often found in nature
  - *Small droplets of suspended virus during aerosol transmission*
  - *Activation of a latent virus from a limited population of cells*
  - *Small volume of inoculum introduced in infection by insect bites*
- How do infections that spread by these routes escape Muller's ratchet?

Avoiding the 'ratchet'

- Subject a more diverse viral population to serial passage
  - *Don't pick a single plaque, pool several plaques*
- More diversity in the replicating population facilitates construction of a mutation-free genome by recombination or reassortment, removing or compensating for mutations that affect growth adversely

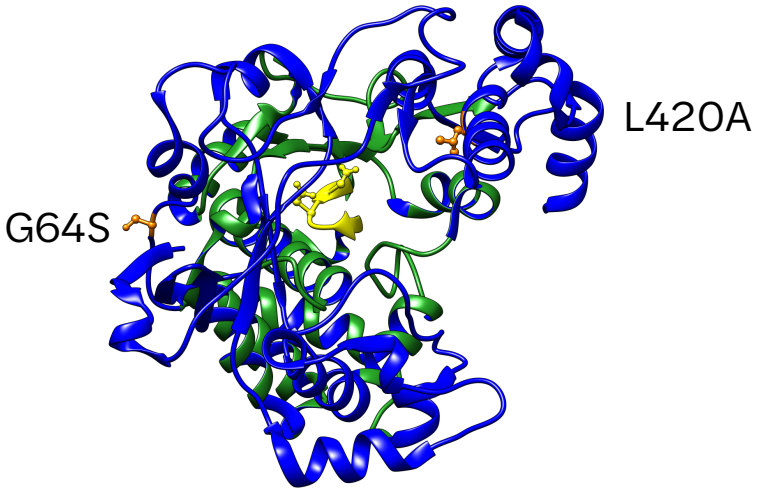


By exchange of genetic information

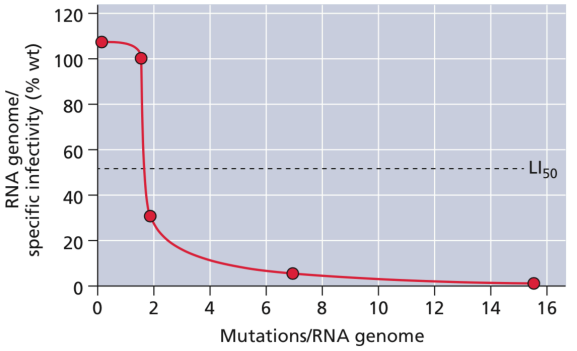


Don’t forget reassortment

By exchange of genetic information

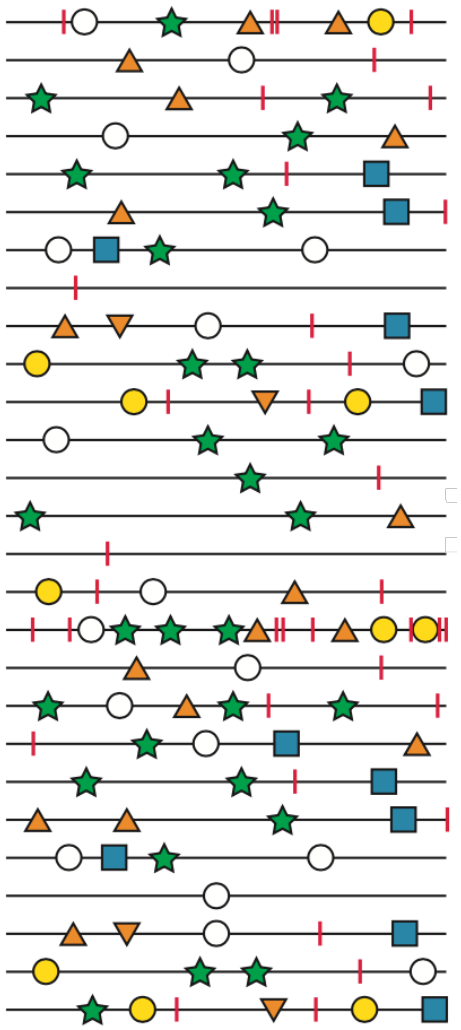


- Poliovirus RdRp L420A is recombination defective
- This mutant exacerbates ribavirin-induced error catastrophe
- Recombination needed to counter error catastrophe



Avoiding the 'ratchet'

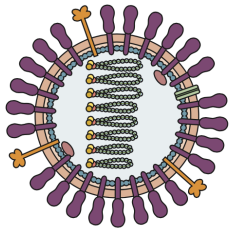
- The message is simple: Diversity of a viral population is important for the survival of individual members
  - *Remove diversity, and the population suffers*



An example of selection: genetic shift & drift

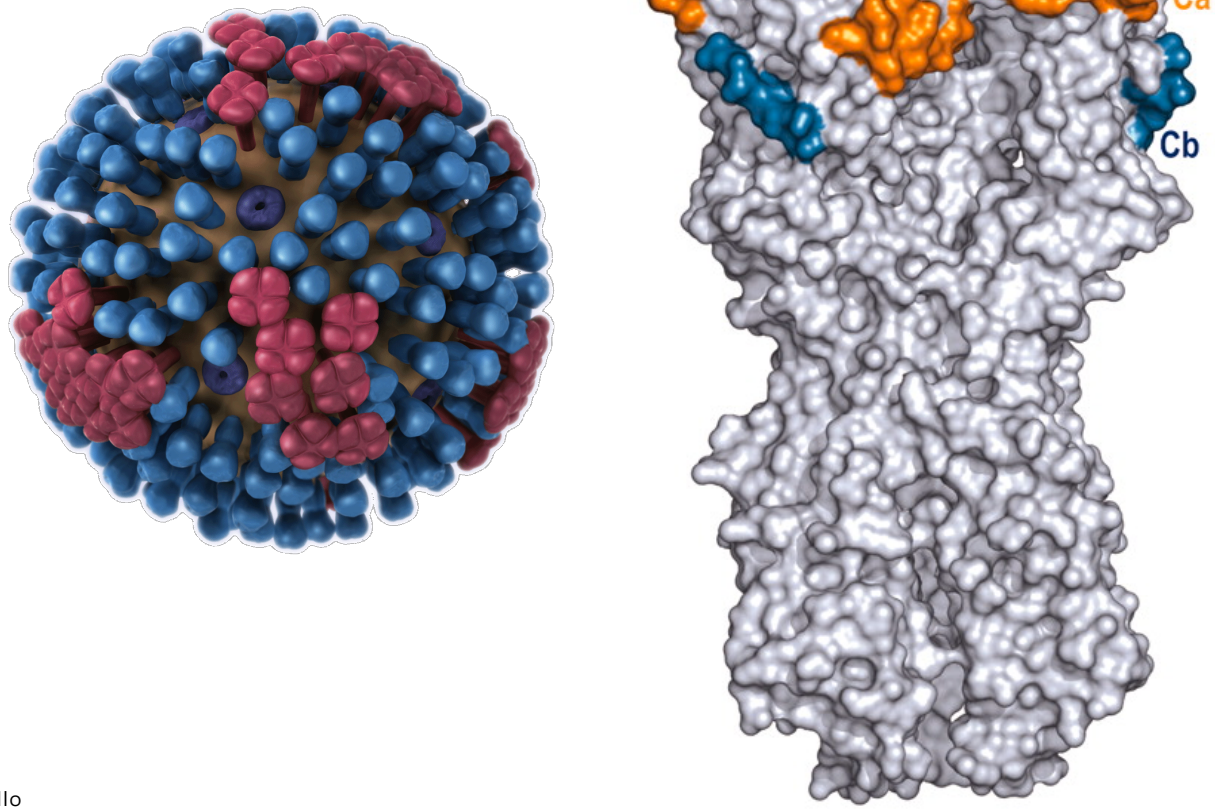
- Selection of viral mutants resistant to elimination by antibodies or cytotoxic T cells inevitable when sufficient virus replication occurs in an immunocompetent individual
- **Drift** - diversity arising from copying errors and immune selection - may occur each time a genome replicates
- **Shift** - diversity arising after recombination or reassortment

Influenza viruses

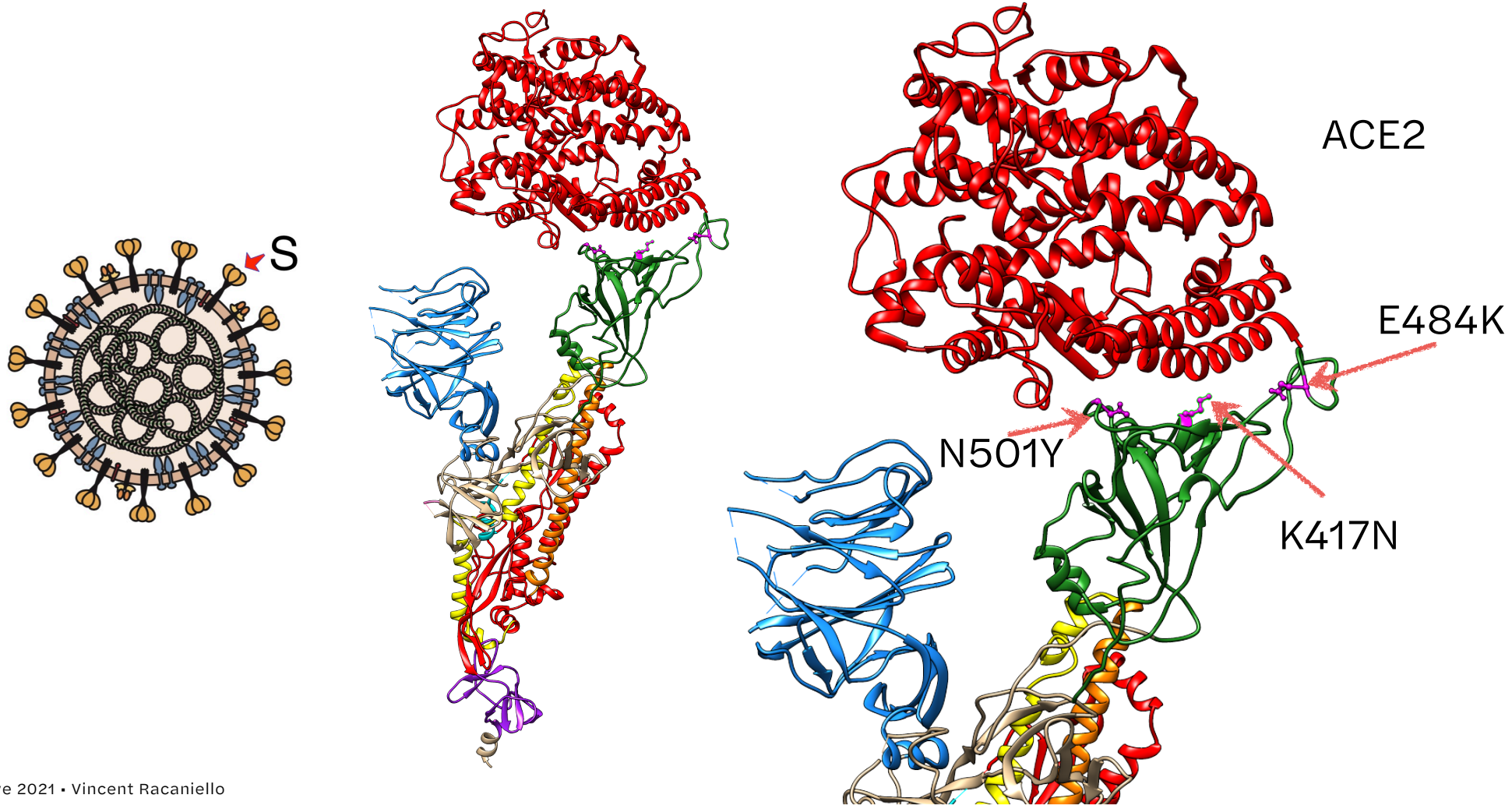


- Influenza A viruses are classified by antigenic composition, by serologic testing of HA and NA
- Combinations of H and N are called HxNy
- x = 1-18; y = 1-11
- H1-17 can infect birds; H1, H2, H3 can infect and transmit between humans

Antigenic drift: Influenza virus

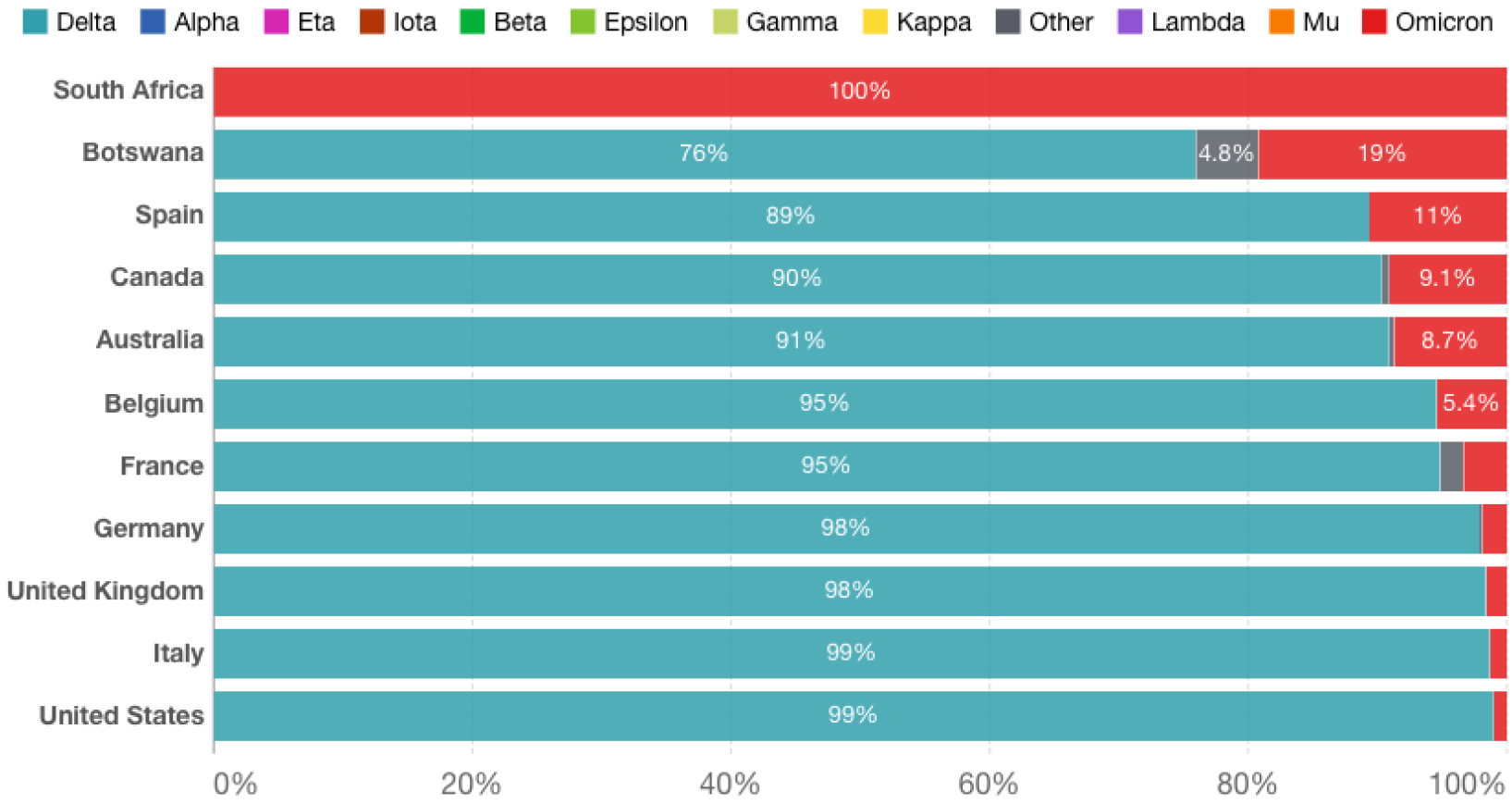


SARS-CoV-2 is undergoing antigenic drift



SARS-CoV-2 sequences by variant, Dec 13, 2021

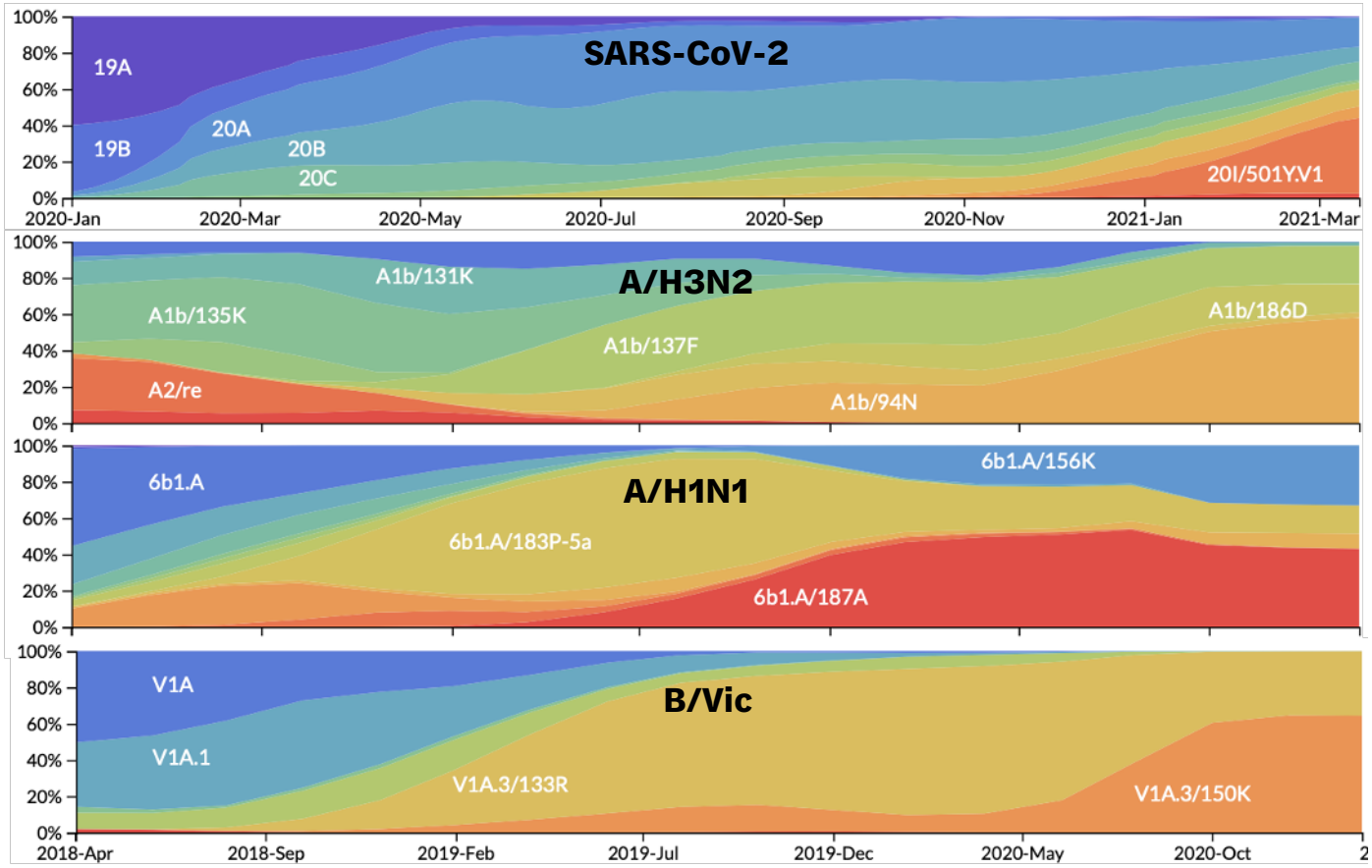
The share of analyzed sequences in the preceding two weeks that correspond to each variant group. This share may not reflect the complete breakdown of cases since only a fraction of all cases are sequenced.



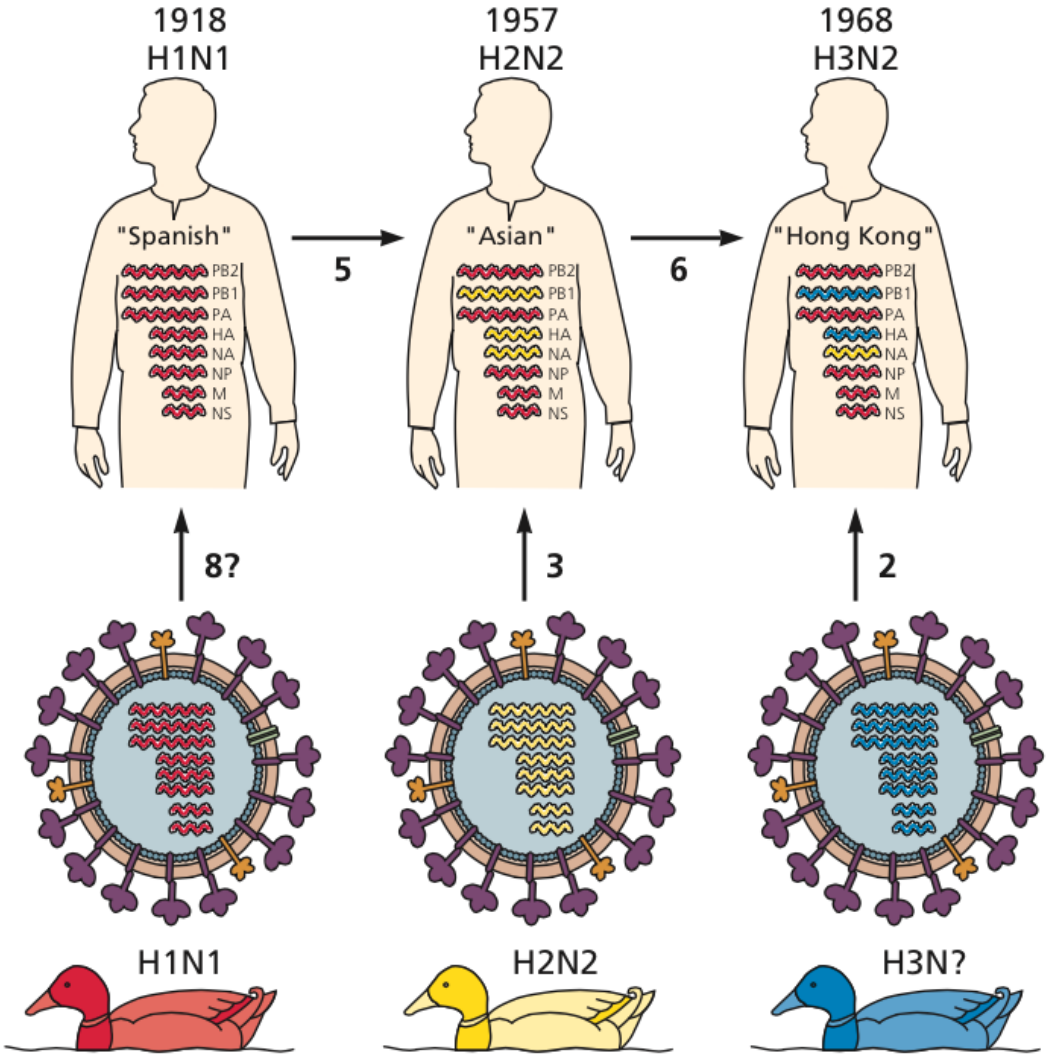
Source: CoVariants.org and GISAID – Last updated 14 December 2021, 20:10 (London time) OurWorldInData.org/coronavirus • CC BY
Note: Recently-discovered or actively-monitored variants may be overrepresented, as suspected cases of these variants are likely to be sequenced preferentially or faster than other cases.

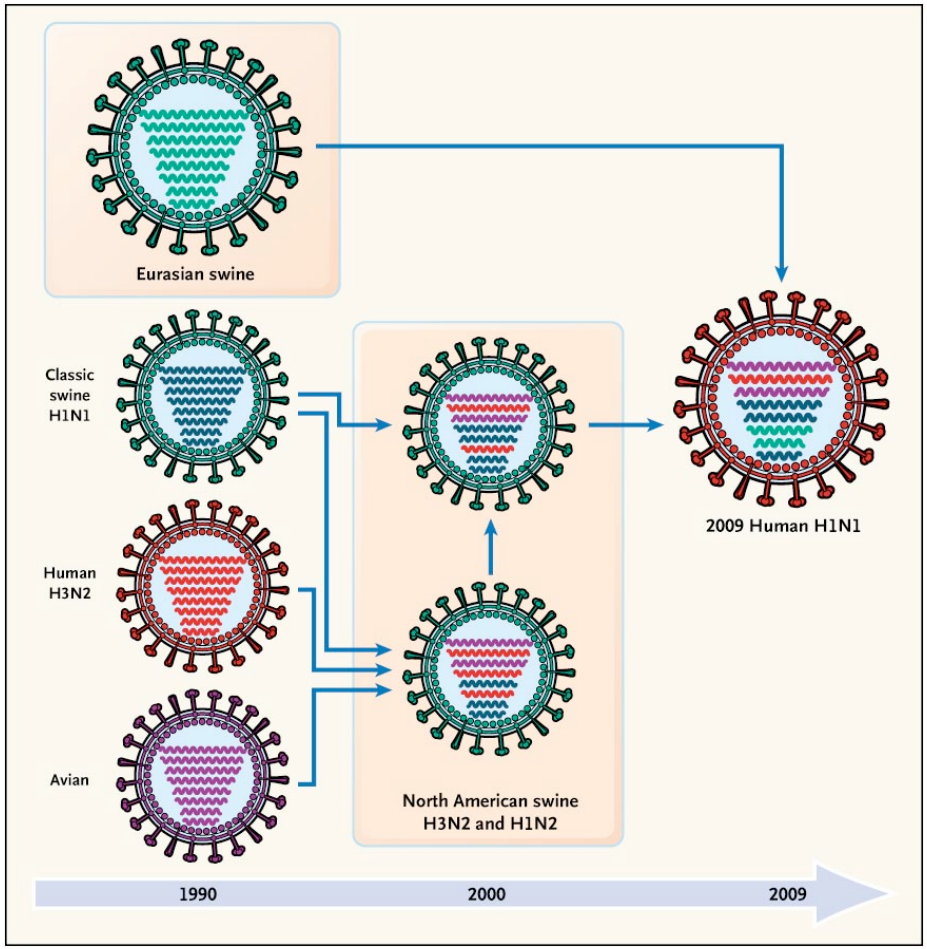
Global variant tracking of influenza virus

Similar clade/lineage replacement



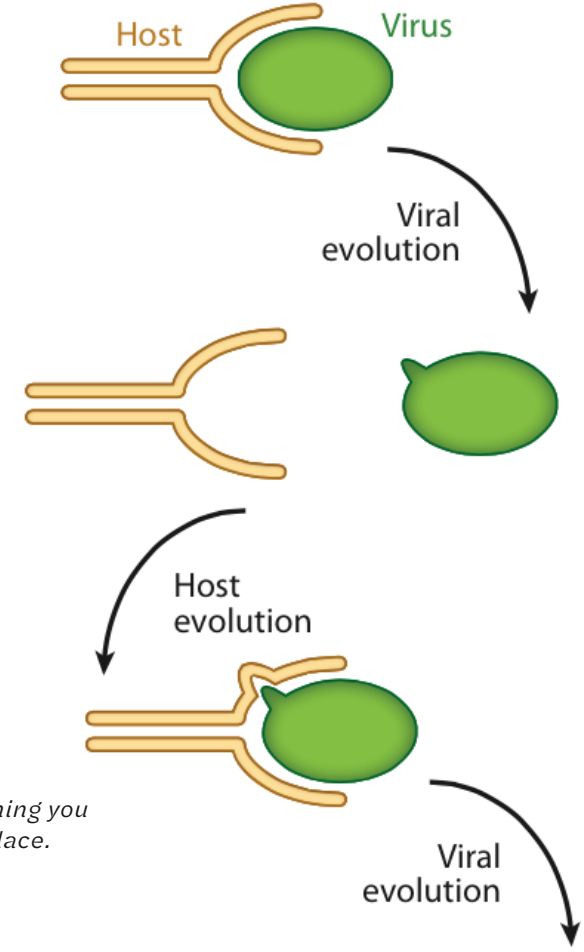
Influenza:
No evidence for increased intrinsic transmissibility





Host-virus arms race

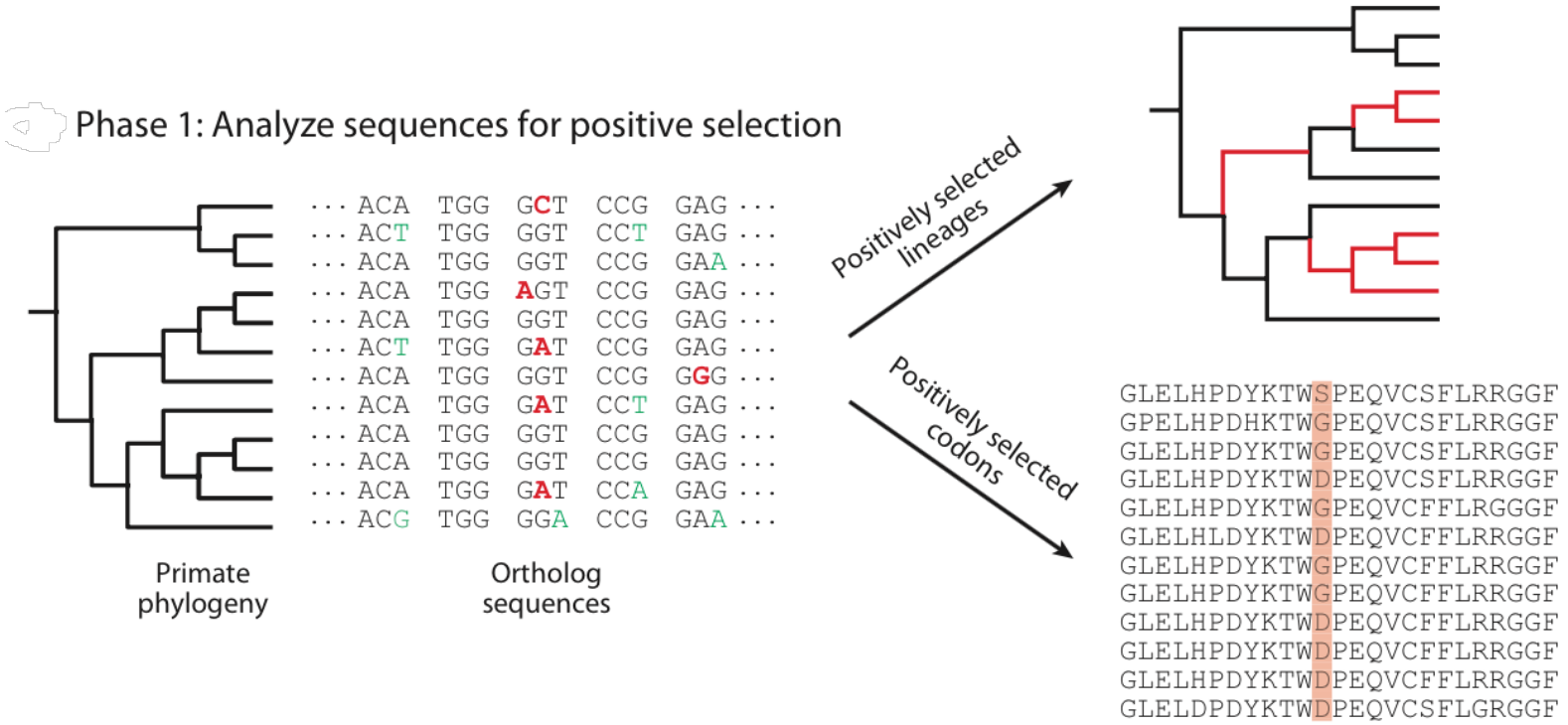
Red Queen conflicts



Around here, it takes all the running you can do just to stay in the same place.
LEWIS CARROLL
Alice in Wonderland

Annu. Rev. Genet. 2012. 46:677–700

Evolution-guided functional analysis of host-virus arms races

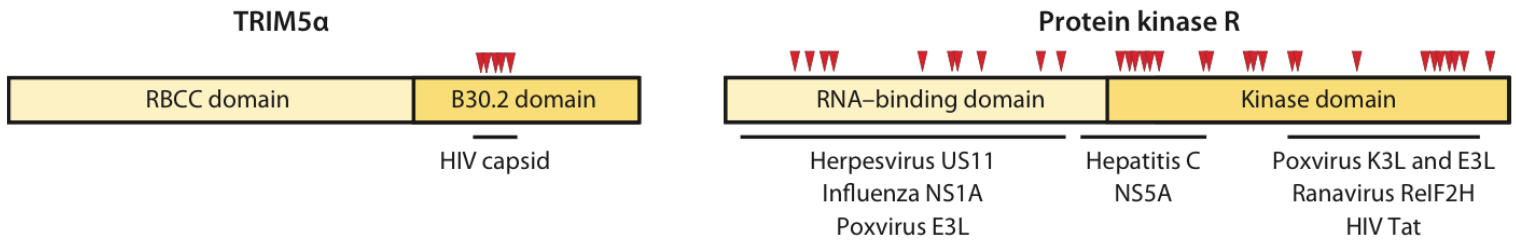


Concept: synonymous and non synonymous changes

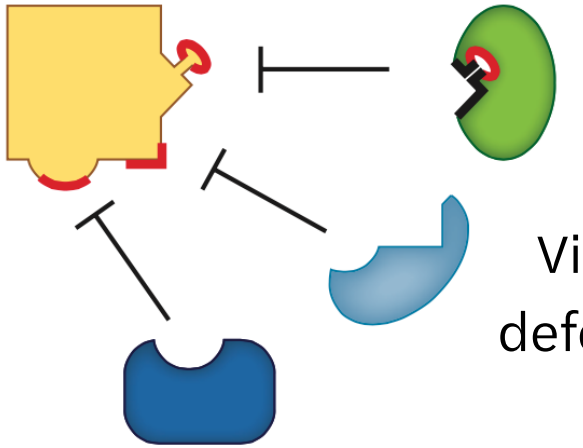
dN/dS

Annu. Rev. Genet. 2012. 46:677–700

Virus-host conflicts have driven evolution of the immune system

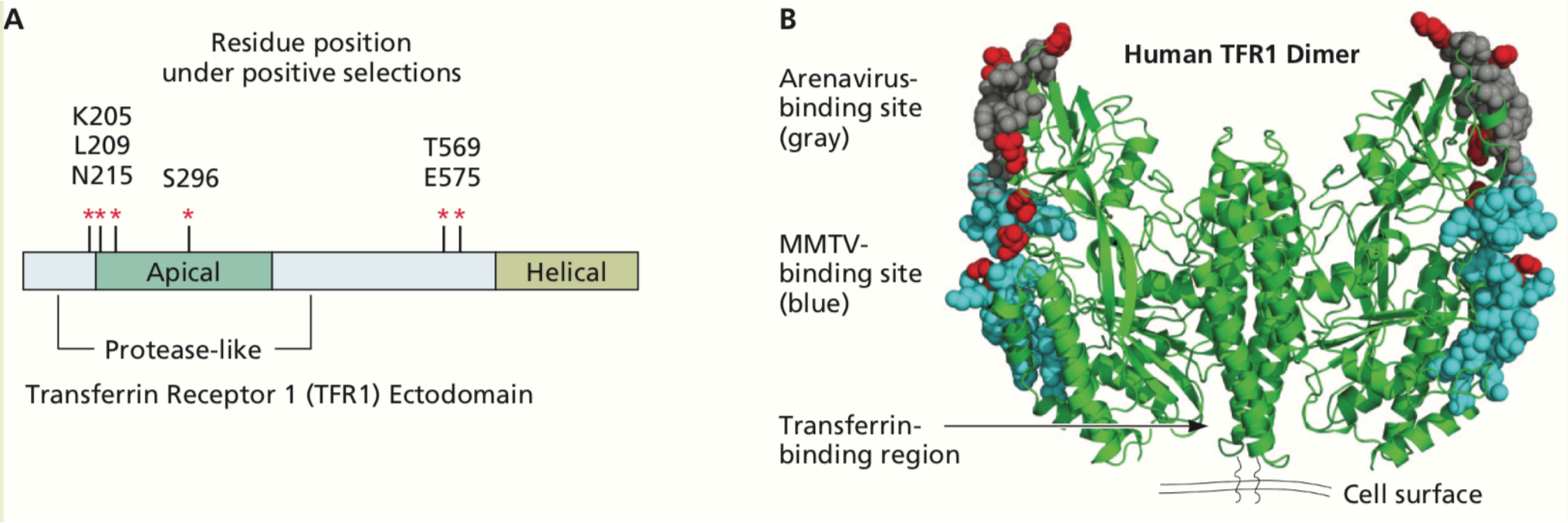


Positively selected amino acids

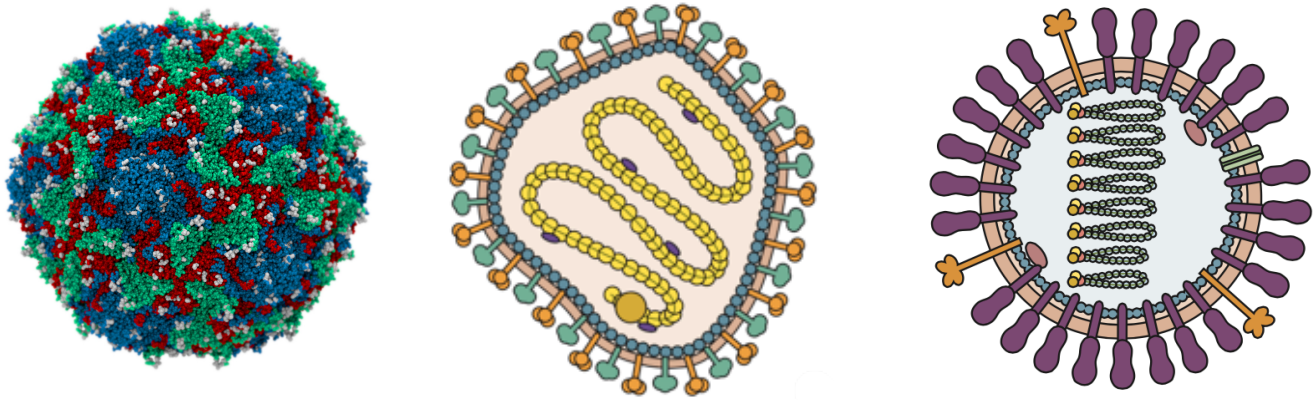


Annu. Rev. Genet. 2012. 46:677–700

TRF1 evolution in rodents shaped by two virus-host races



Despite this genome diversity...



- There are only 3 serotypes of poliovirus (>150 rhinoviruses)
- One measles serotype, continuous influenza variation
- Poliovirus and measles viruses *can* undergo antigenic change, but rarely in humans

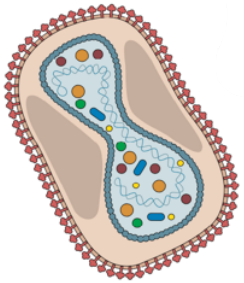
Selection: Is virulence a positive or negative trait?

- *Positive trait*: increased virulence is a consequence of high viral loads, facilitating transmission
- *Negative trait*: increased virulence reduces transmissibility because hosts die faster, reducing exposure to uninfected hosts
- *Neutral trait*: Increased or decreased virulence a consequence of a different selected phenotype
- Probably both: there are many virulent and avirulent viruses

An experiment in the evolution of virulence

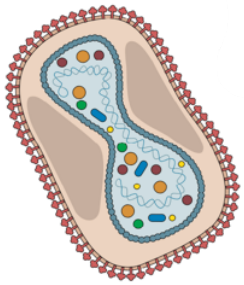
- In 1859, the European rabbit was introduced to Australia for hunting purposes
- Lacking natural predators, it reproduced to plague proportions in a short time

Evolution of virulence



- Myxoma virus released in Australia in the 1950s in an attempt to rid the continent of the rabbits
- Natural host of myxoma virus is the cottontail rabbit
- Virus spread by mosquitoes; infected rabbits develop superficial warts on their ears
- European rabbits are a different species, infection is 90-99% fatal

Evolution of virulence



- In the first year, the released virus was efficient in killing rabbits with a 99.8% mortality rate
- After the second year the mortality dropped to 30%
- Rate of killing was lower than the reproductive rate of the rabbits, and hope for 100% eradication was dashed

Evolution of virulence

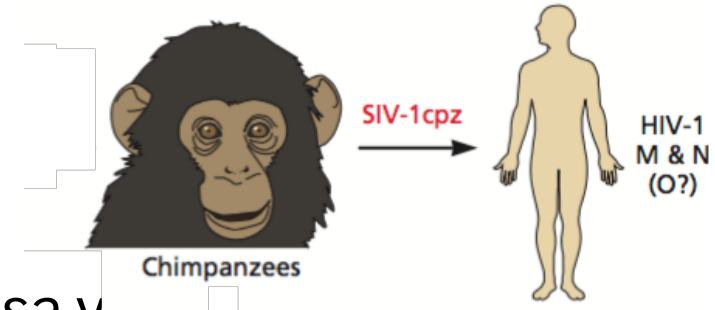
- Both rabbits and viruses produce large numbers of offspring
- Virus variants were selected that kill fewer rabbits and extend the life of lethally infected rabbits so that the virus could overwinter and spread in spring mosquitoes
- The rabbits evolved to become more resistant or tolerant of the virus
- As predicted for an evolving host coming to an equilibrium with the pathogen


nature **NEWS** · 22 FEBRUARY 2018

Doubts raised over Australia's plan to release herpes to wipe out carp

Warm water may render the virus ineffective against the invasive fish, say researchers.

Evolution of viral virulence in humans?



- Experience with Lassa virus, Ebolavirus, HIV-1: animal-human virus transfers tend to be virulent
- But viruses from older jumps (measles, poliovirus) are less virulent
- What happened in the meantime?
- SARS-CoV-2: no solid data yet on changes in virulence

Nevertheless, we are obsessed with increased viral virulence

- Ebolavirus is mutating, will go airborne (Osterholm, NY Times, 11 Sept 2014)
- Ebolavirus, Lisa Henley: "Is it getting better at replicating as it goes from person to person?" (*Ebola Wars*, Richard Preston, *New Yorker*)
- Peter Hotez, *NYT* Op-Ed 8 April 2016: "There are many theories for Zika's rapid rise, but the most plausible is that the virus mutated from an African to a pandemic strain a decade or more ago and then spread east across the Pacific from Micronesia and French Polynesia, until it struck Brazil."
- It's easy to blame mutation - but usually there are other explanations (e.g. poliovirus, see 'Emerging Viruses')
- SARS-CoV-2 variants of concern suggested to have increased virulence: unproven

‘Don’t go to Wuhan, don’t leave Wuhan’: Coronavirus could mutate and spread further, China officials warn

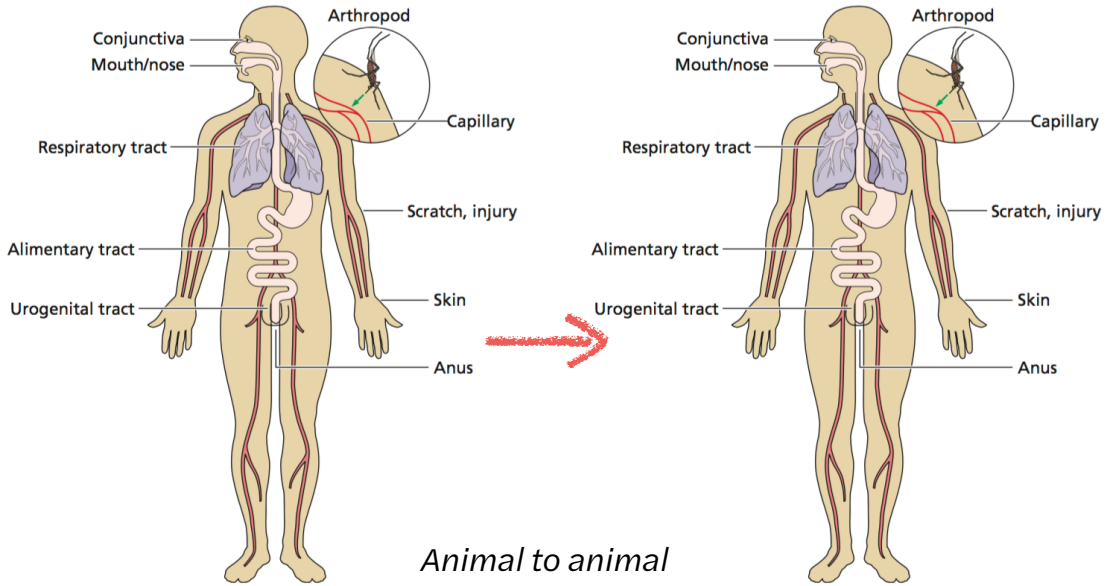
Some 440 cases confirmed so far as disease reported in Thailand, Japan, South Korea and United States

China warns virus could mutate and spread as death toll rises

Updated / Wednesday, 22 Jan 2020 **11:45**

“Evidence has shown that the disease has been transmitted through the respiratory tract and there is the possibility of viral mutation” —National Health Commission Vice Minister Li Bin

We have no data on the effect of evolution on viral virulence in humans



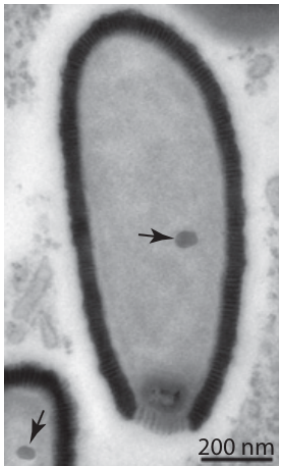
Animal to animal

Transmission to a new host is a major selective force for viral evolution

SARS-CoV-2 variants of concern have increased FITNESS

- Fitness is the ability of the virus to reproduce in the host
- Improved fitness can be conferred by changes in many processes, including
  - Reproduction
  - Transmission
  - Immune evasion
- What confers increased fitness to VOC is not known

The origin of viruses



Oldest viral stocks:

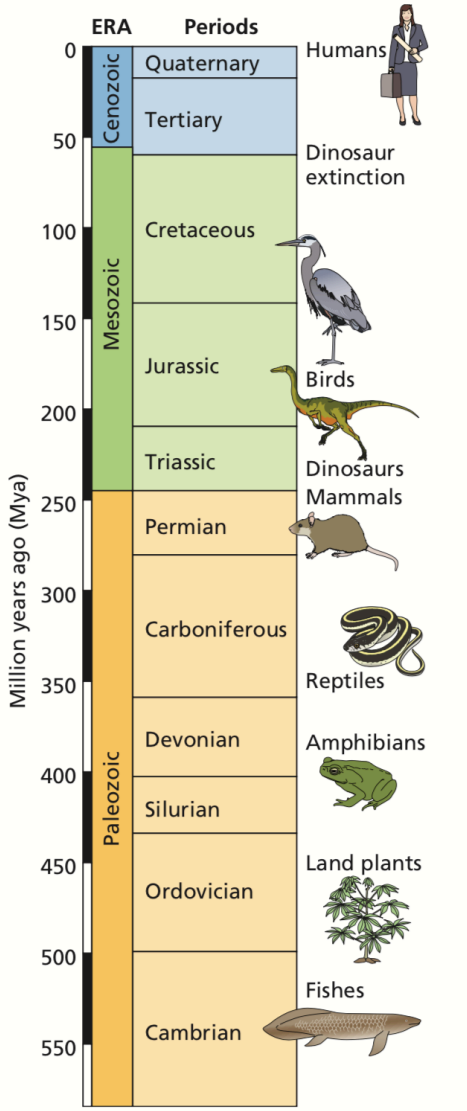
1918 influenza virus

Pithovirus sibericum

(30,000 y)

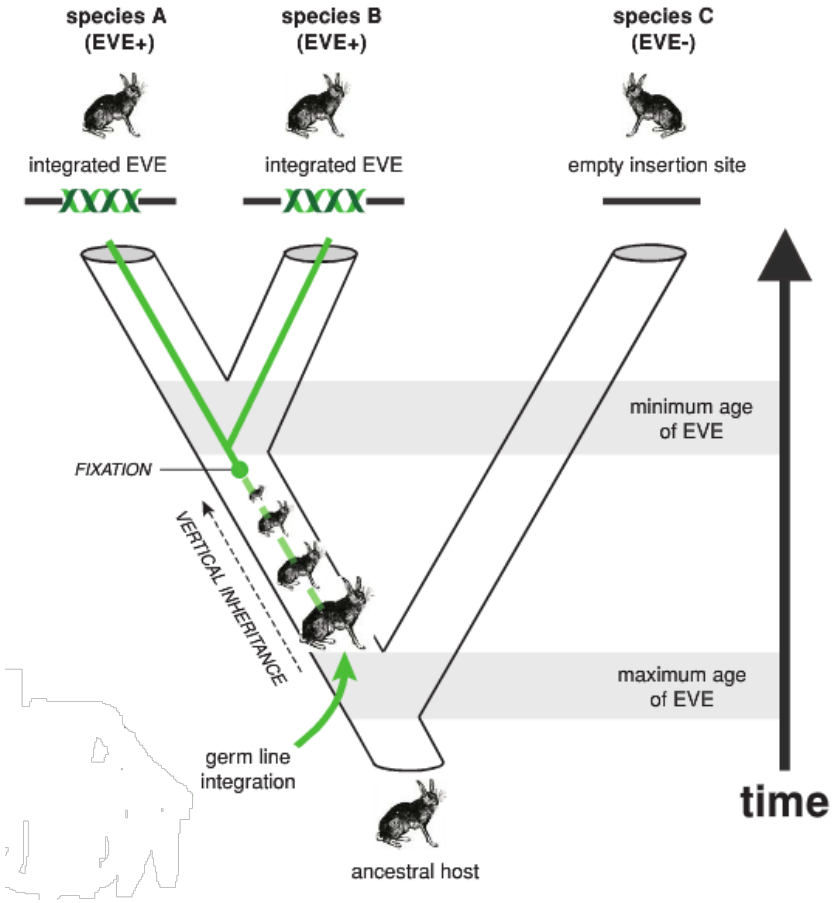
Origins of DNA viruses

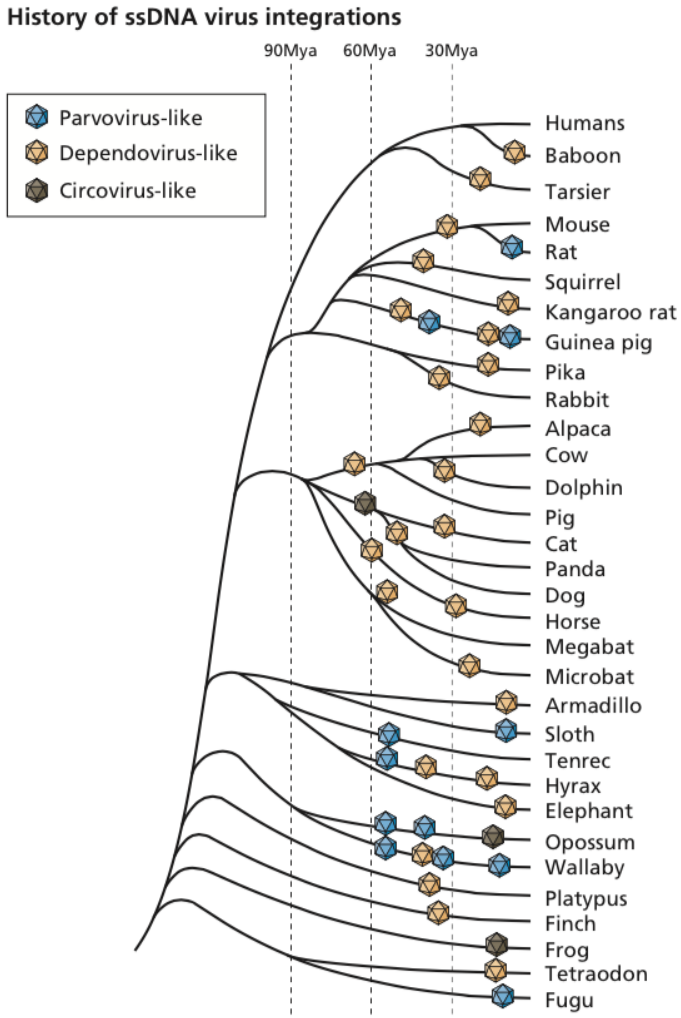
Molecular clocks: By relating timescale of herpesviral genome evolution with that of hosts, believe that three major groups of herpesviruses (alpha, beta, gamma) arose ~180-220 million years ago



Endogenous viruses - retrovirus and otherwise

Phylogenomics

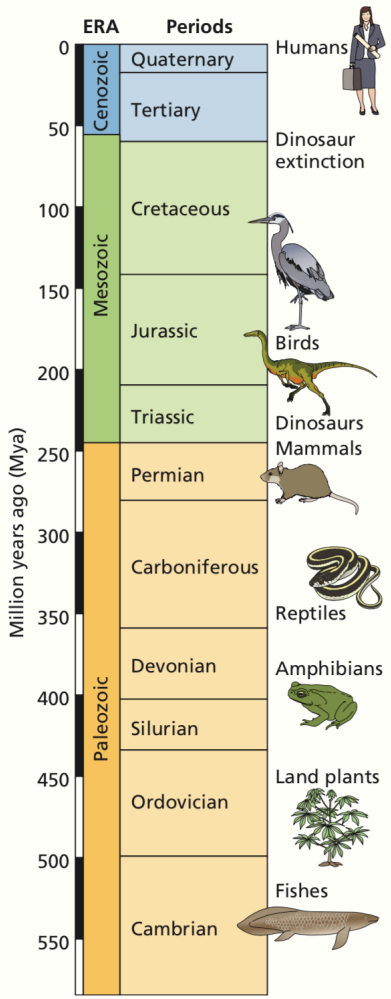




How old are viruses?

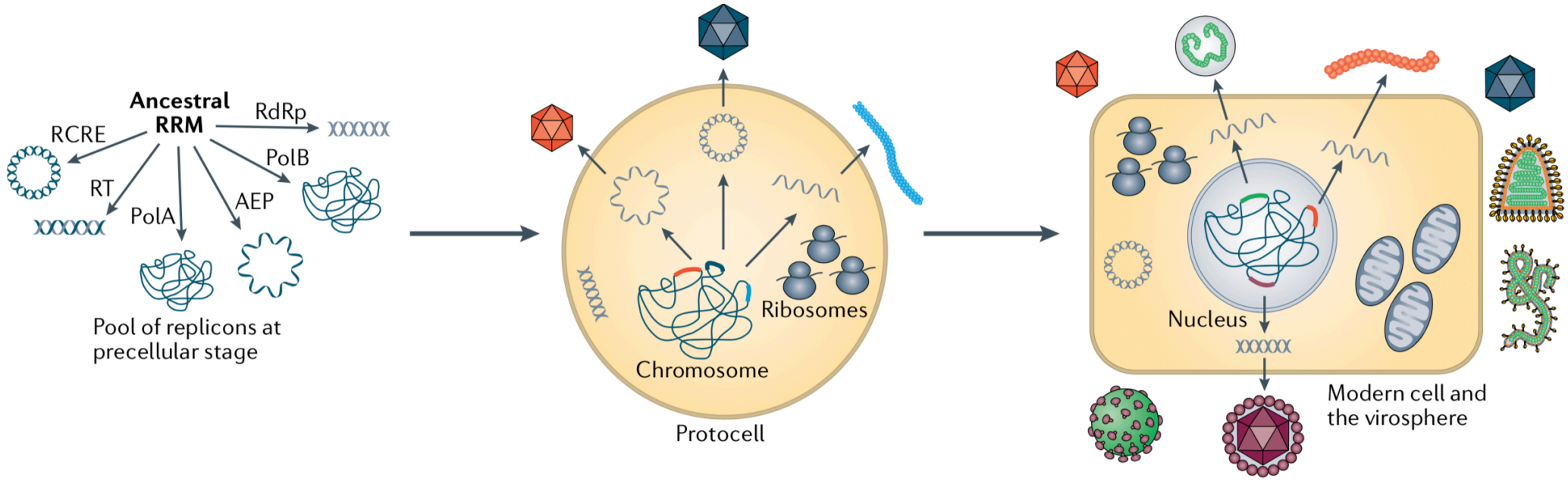
Orthoceras, a nautiloid cephalopod, 488 Mya

Nobu Tamura (http://spinops.blogspot.com)



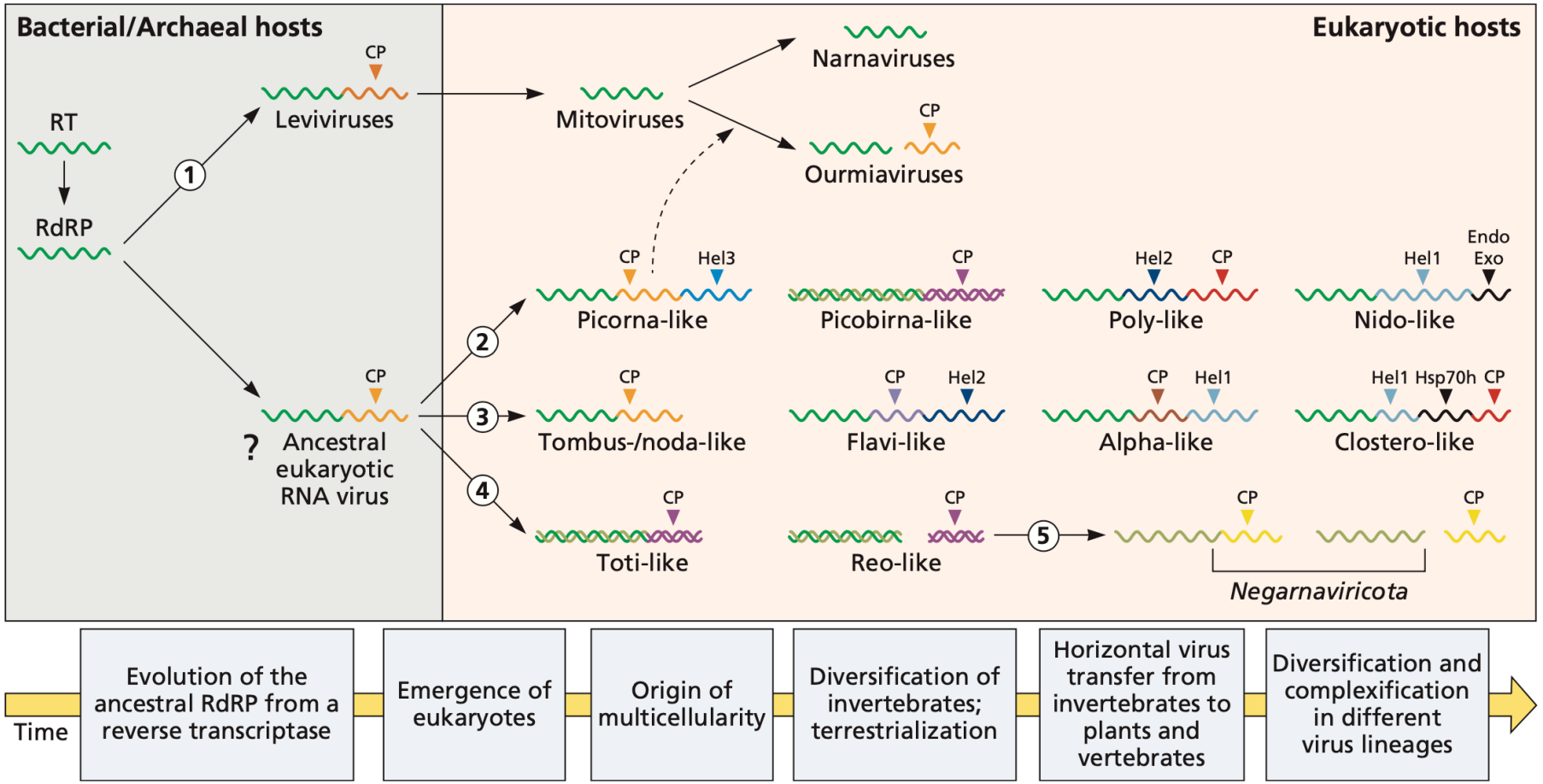
- Estimates of molecular evolution suggest marine origin of some retroviruses >450 Mya, Ordovician period
- Likely originated billions of years ago - before cells?

Origin of viruses

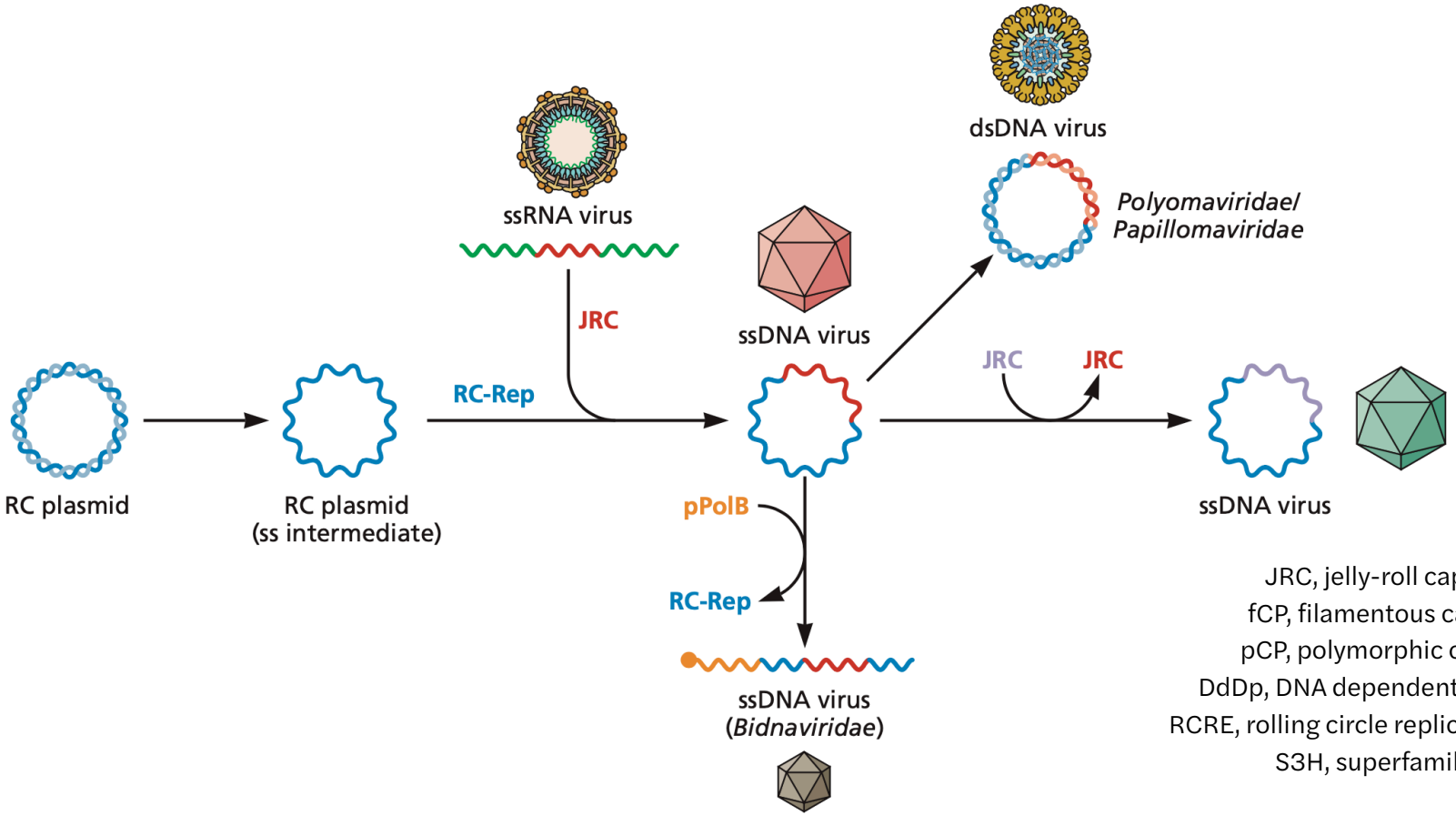


Nature Reviews Microbiology 17, 449–458 (2019)

Scenario of RNA virus evolution



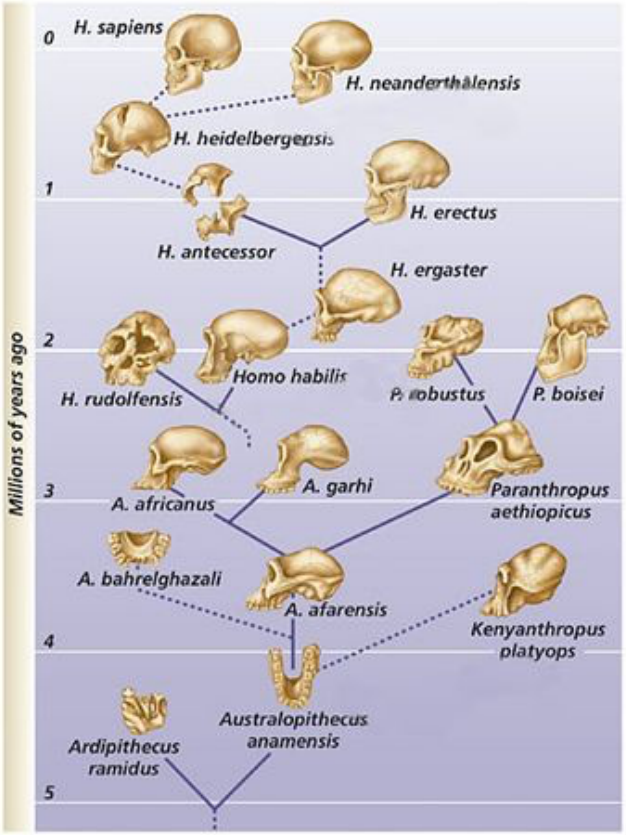
Multiple origins of ssDNA viruses



JRC, jelly-roll capsid protein
fCP, filamentous capsid protein
pCP, polymorphic capsid protein
DdDp, DNA dependent DNA polymerase
RCRE, rolling circle replication endonuclease
S3H, superfamily 3 helices

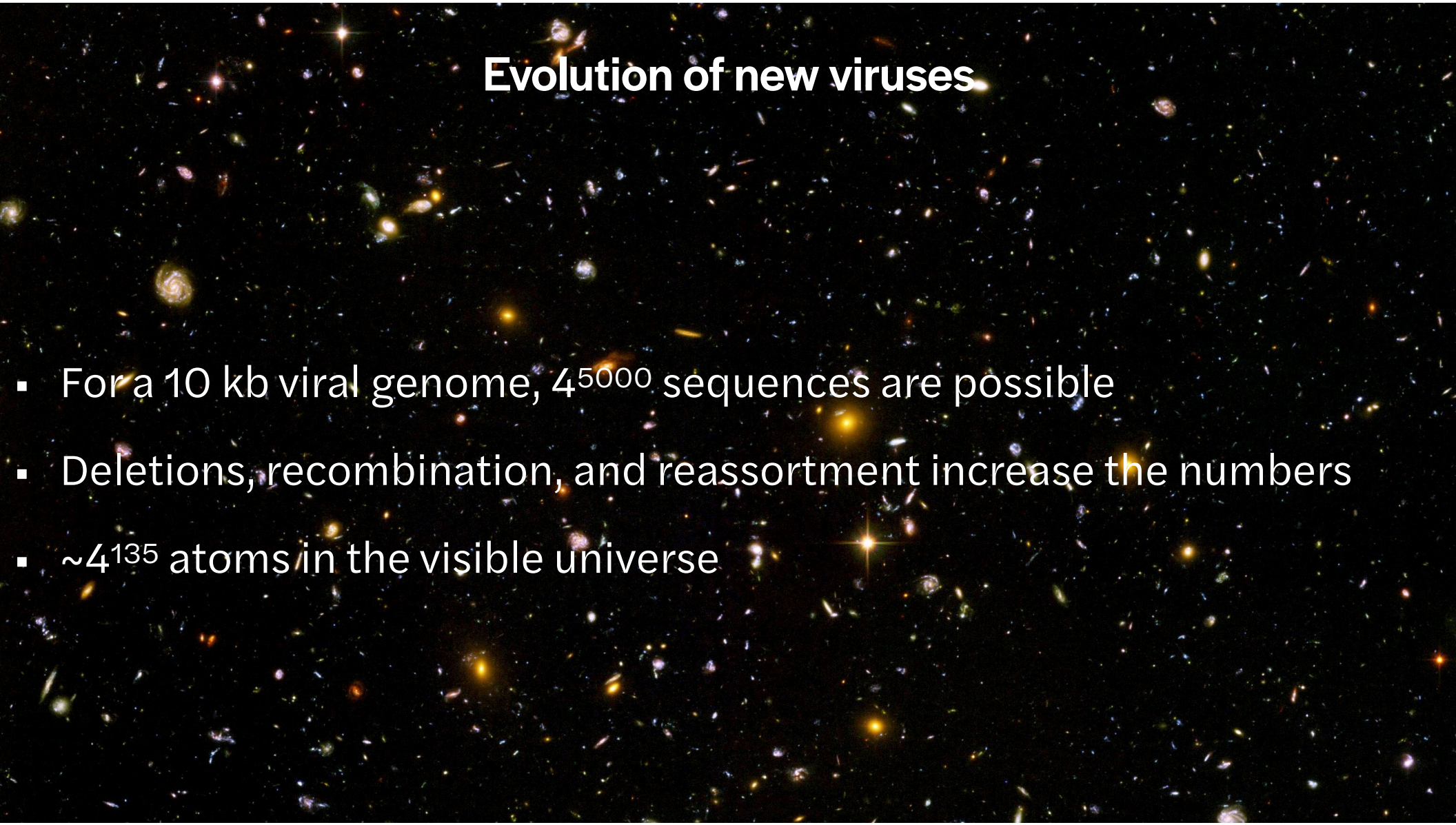
Human viruses

- All known types of viruses likely evolved long before humans appeared on Earth
- All human viruses have therefore evolved from animal viruses



Evolution of new viruses

- Assumption: new viruses can only arise from viruses that are now in existence, not *de novo*
- What is the number of all possible mutations of a viral genome?
- Sequence comparisons of several RNA virus genomes have demonstrated that *well over half* of all nucleotides can accommodate mutations



Evolution of new viruses

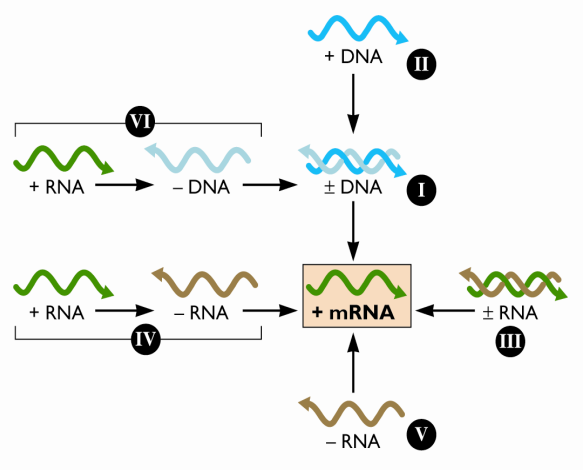
- For a 10 kb viral genome, 4^{5000} sequences are possible
- Deletions, recombination, and reassortment increase the numbers
- ~4^{135} atoms in the visible universe

The fundamental properties of viruses constrain and drive evolution

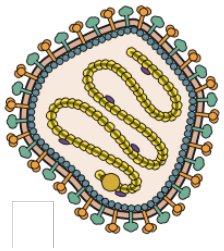
- Despite many rounds of replication, mutation, selection, we can recognize a herpesvirus or influenza virus genome by sequence analysis
- Viral populations often maintain master or consensus sequences, despite opportunities for extreme variation
- How is stability maintained?

Constraining viral evolution

- Extreme alterations in viral consensus genome do not survive selection
- The viral genome is one constraint
  - *DNA cannot become RNA, or vice versa*
  - *Replication - interaction with host proteins, replication signals*
  - *mRNA synthesis signals, poly(A) addition, processing*
  - *RNA structure, codon usage,*

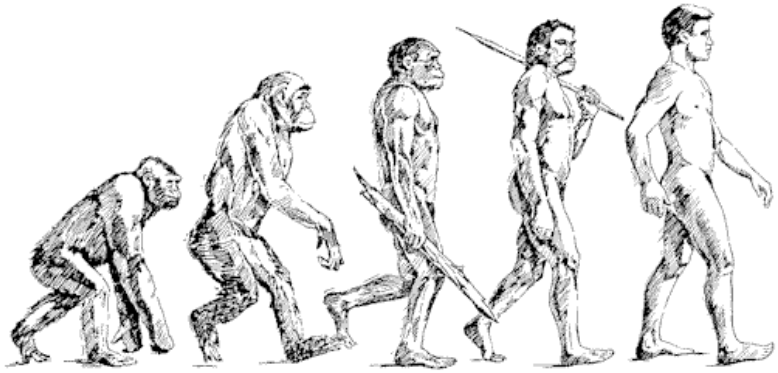


- Physical nature of capsid
  - *Icosahedral capsids: defined internal space, fixes genome size*

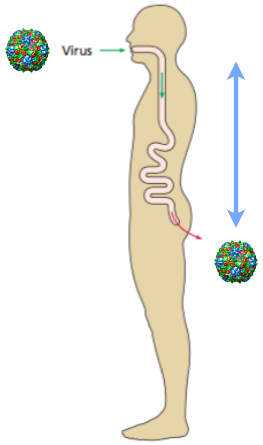


- Selection during host infection
  - *A mutant too efficient in bypassing host defenses will kill host, have the same fate as one that does not sufficiently replicate*

Food for thought



8 million years



5 days

Imagine what a virus can do with 8 million years

Next time: Emerging viruses