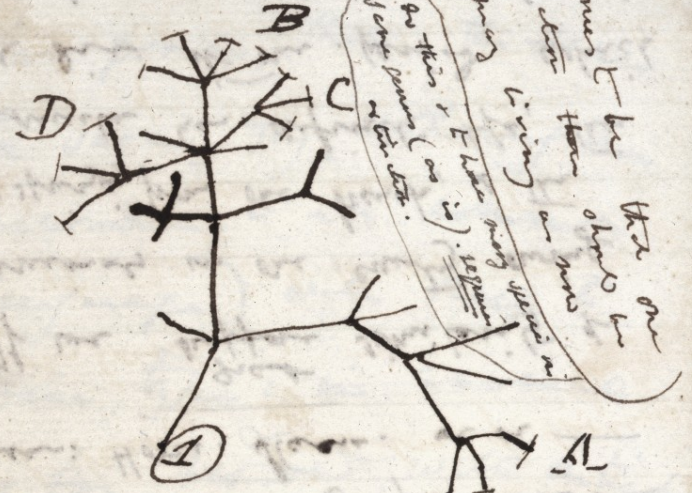
Evolution

Lecture 21
Biology W3310/4310
Virology
Spring 2016

Anything produced by evolution is bound to be a bit of a mess.
Sydney Brenner

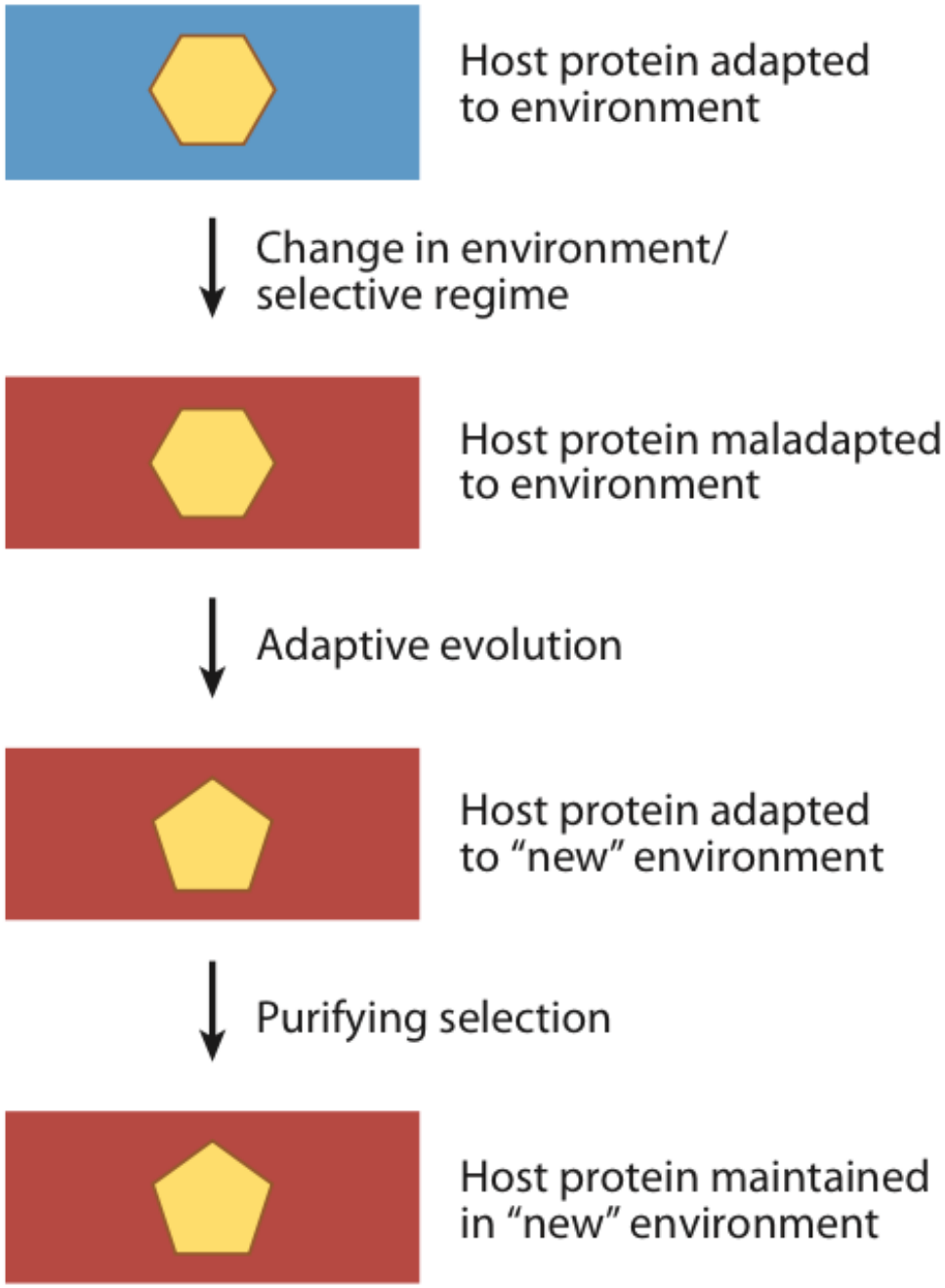
Around here, it takes all the running you can do just to stay in the same place.
Lewis Carroll
Alice in Wonderland

I think

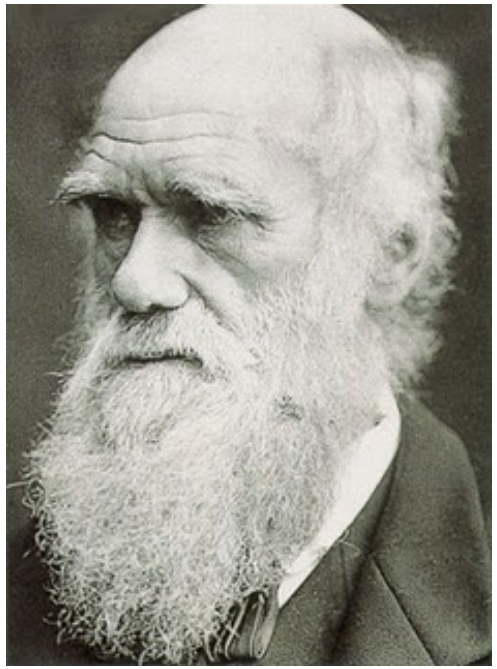


Then between A & D. Chasms
sort of relation. C & B. The
first gradation, B & D
rather greater distinction
Then genera would be
formed. — bearing relation

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
Host protein maintained
in "new" environment

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 in the US, HCV, Ebolavirus, Zika virus
- Regular bouts every year with influenza and common cold viruses
- Drug resistant HIV

Simple fact: viruses evolve 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

Principles of Virology, ASM Press

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!

TIME

The Ebola Virus Is Mutating, Say Scientists

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

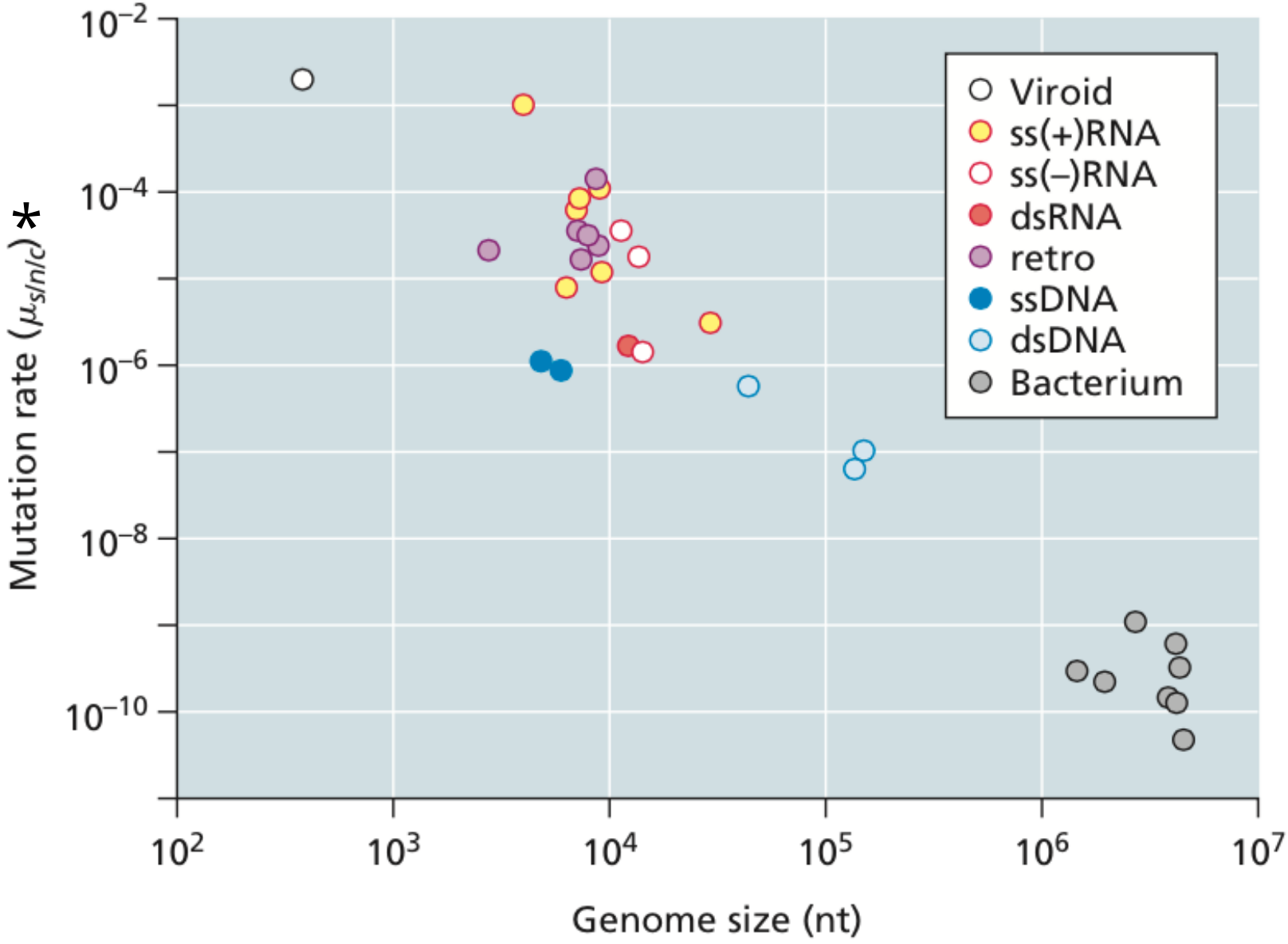
A health care worker, right, takes the temperatures of school children for signs of the Ebola virus before they enter their school in the city of Conakry, Guinea, Monday, Jan. 19, 2015

RNA viruses

- Lack of proofreading activity in RNA dependent RNA polymerase: high error frequencies (1 misincorporation / 10^3 - 10^4 nt polymerized)
- Average error frequency: 1 in 10^4 or 10^5 nucleotides polymerized
- In a 10 kb RNA virus genome, a mutation frequency of 1 in 10^4 results in about 1 mutation per genome

DNA viruses

- Genome replication not as error prone as RNA viruses
- Proofreading
- Most DNA viruses generate less diversity, evolve slower than RNA viruses



*substitutions/nucleotide/generation

Principles of Virology, ASM Press

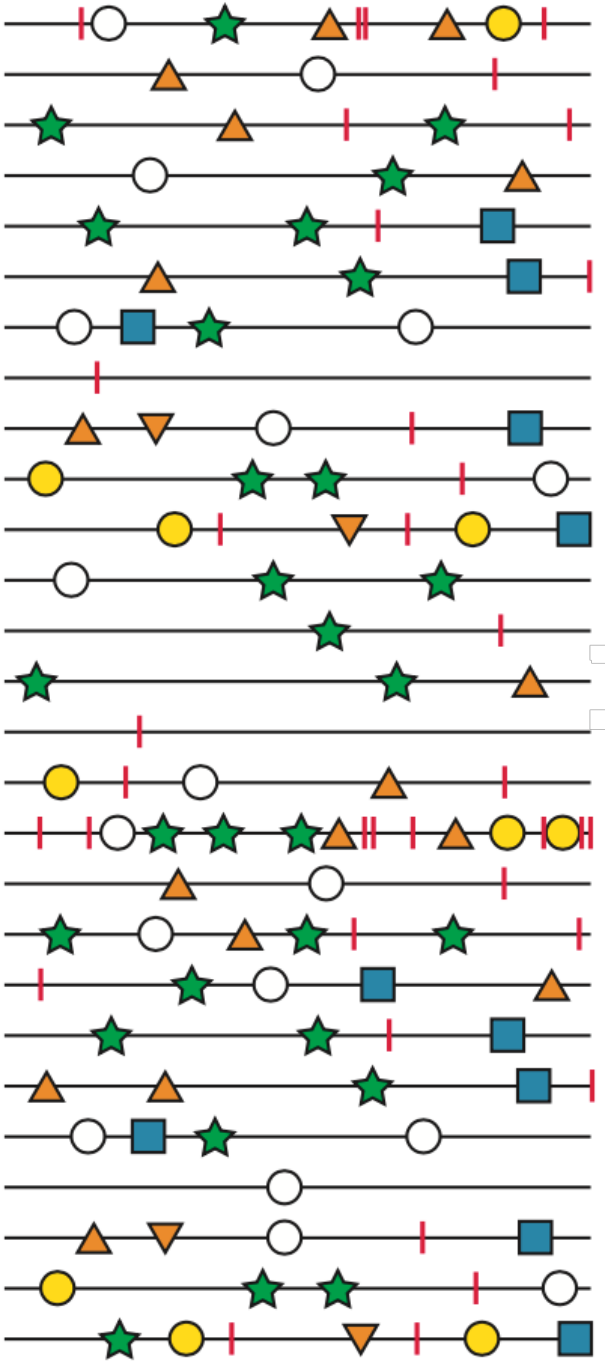
The quasispecies concept

- 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*

Viral quasispecies



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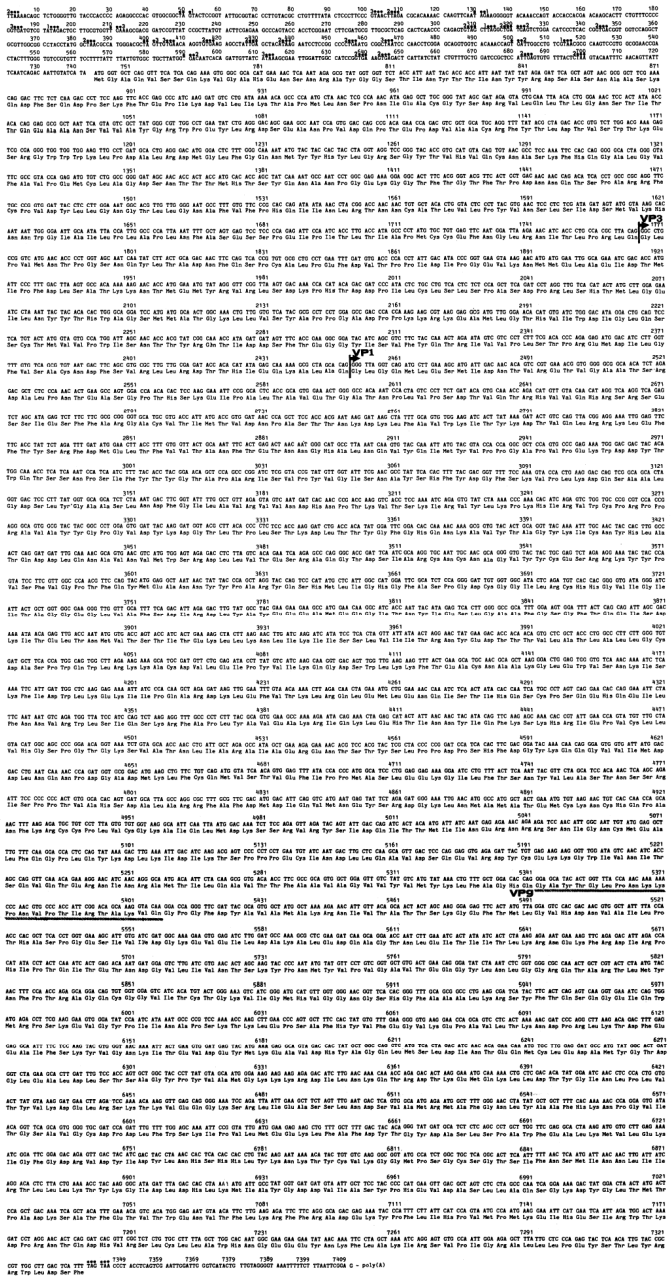


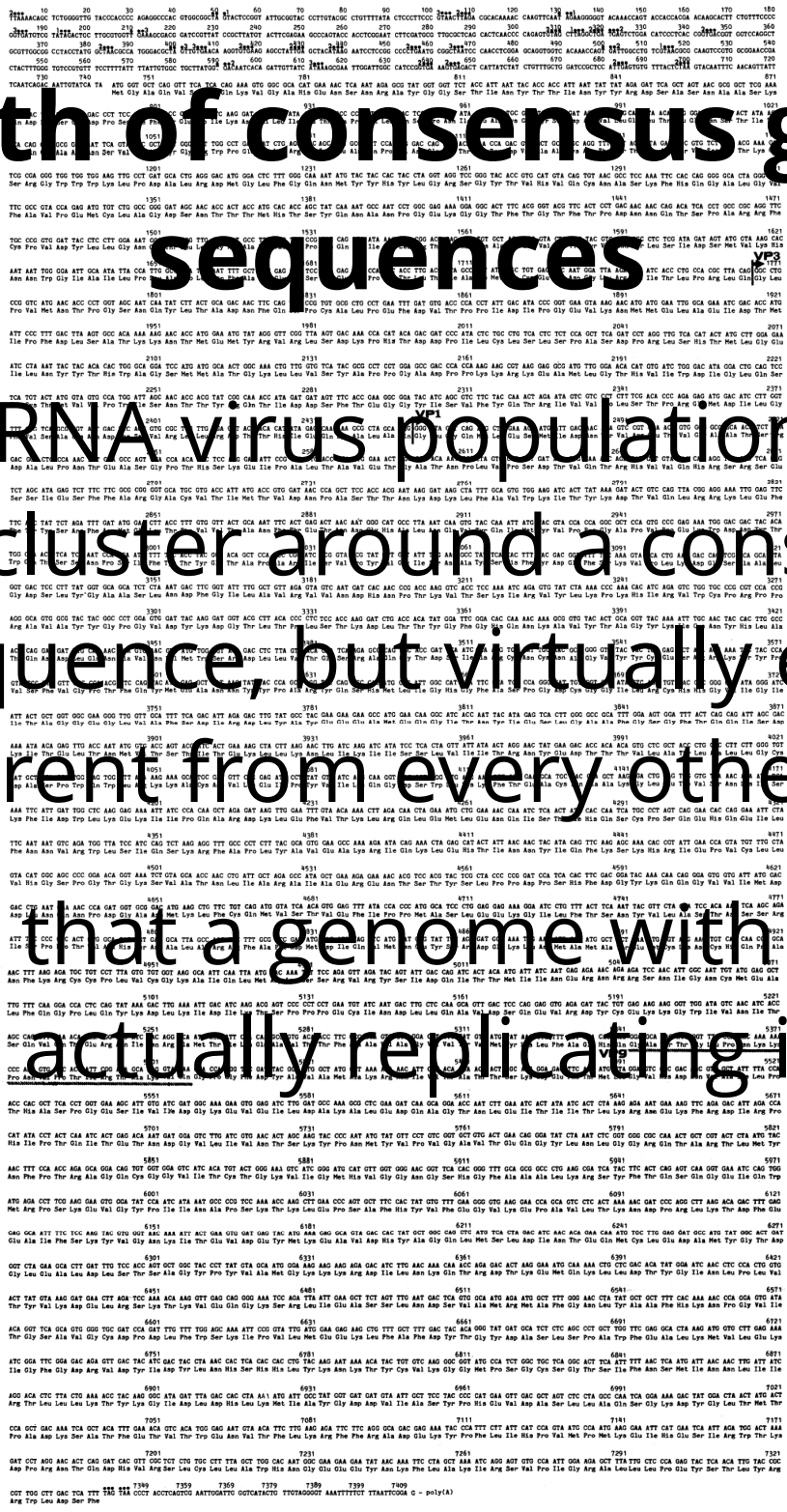
Principles of Virology, ASM Press

Quasispecies effects

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

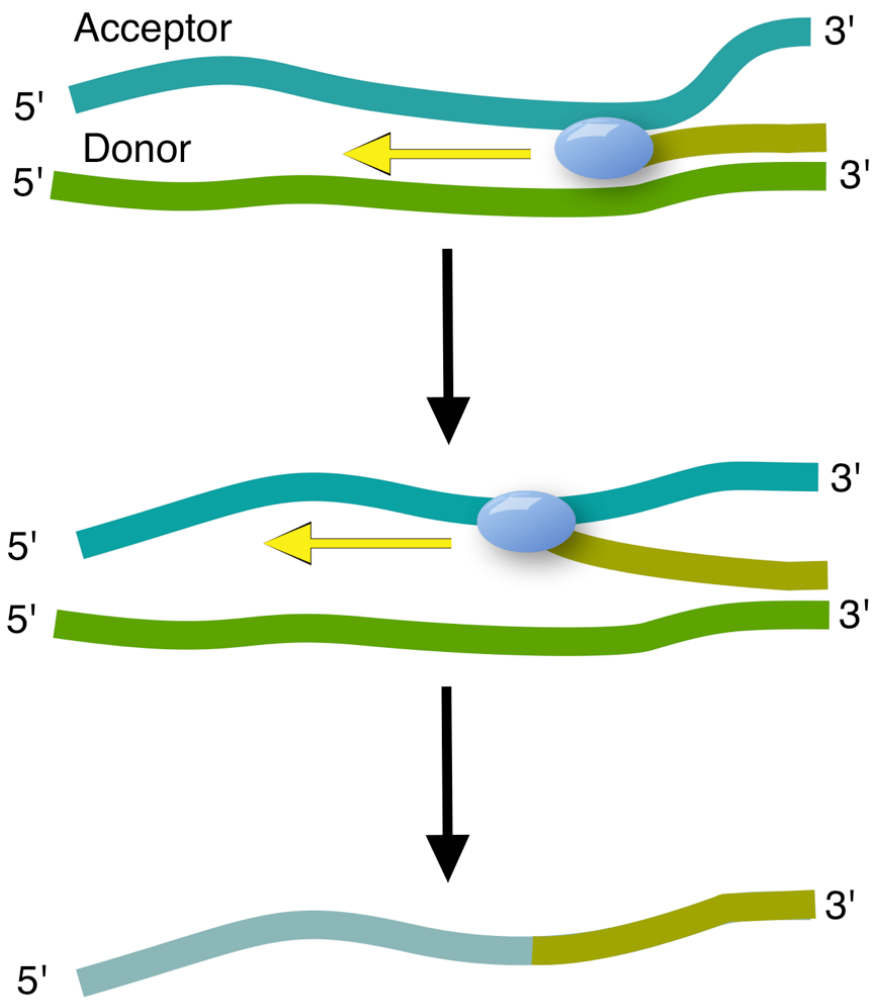
The myth of consensus genome sequences

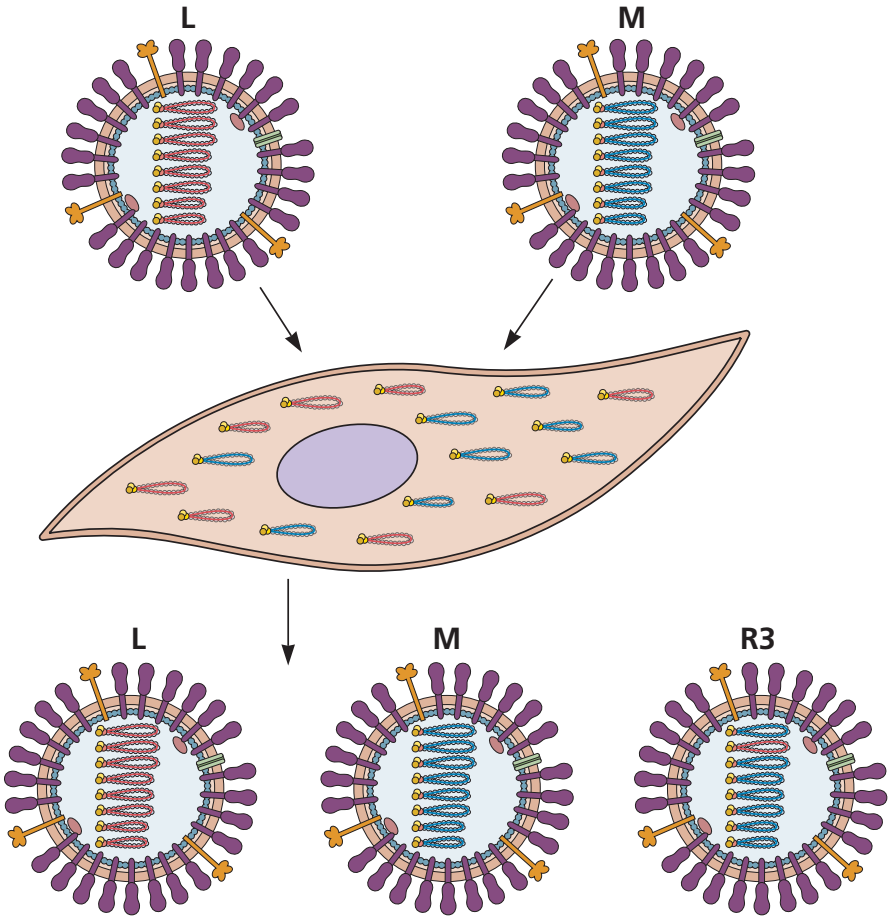
- For a given RNA virus population, the genome sequences cluster around a consensus or average sequence, but virtually every genome can be different from every other
- It is unlikely that a genome with the consensus sequence is actually replicating in the population



Quasispecies

Variation further generated by recombination and reassortment





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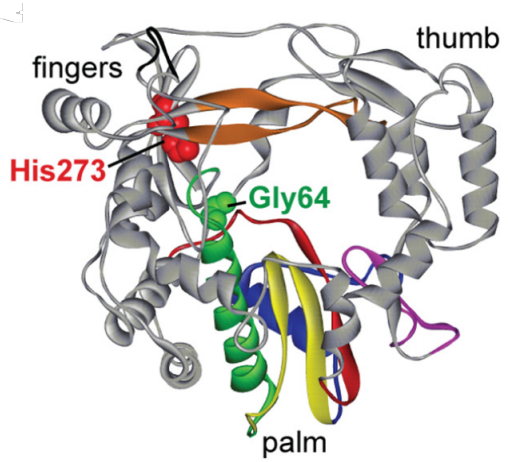
Selection

- *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 only the selected 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

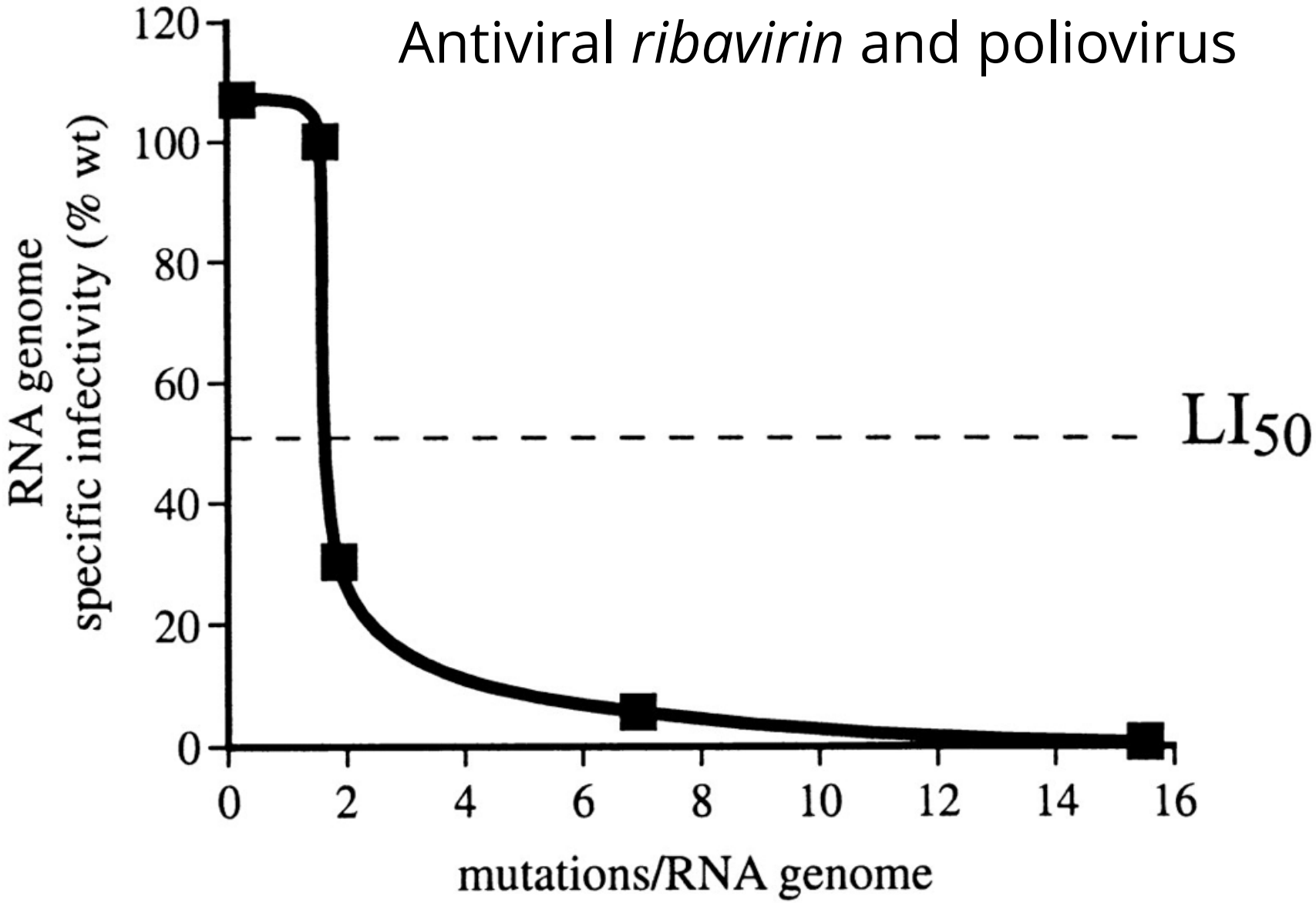
- Mutation is a powerful advantage, but selection and survival balances genetic fidelity and mutation rate
- This limit is called the **error threshold**
  - Exceed it: loss of infectivity
  - Below it: cannot produce enough mutations to survive selection
- RNA viruses: evolve close to their error threshold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

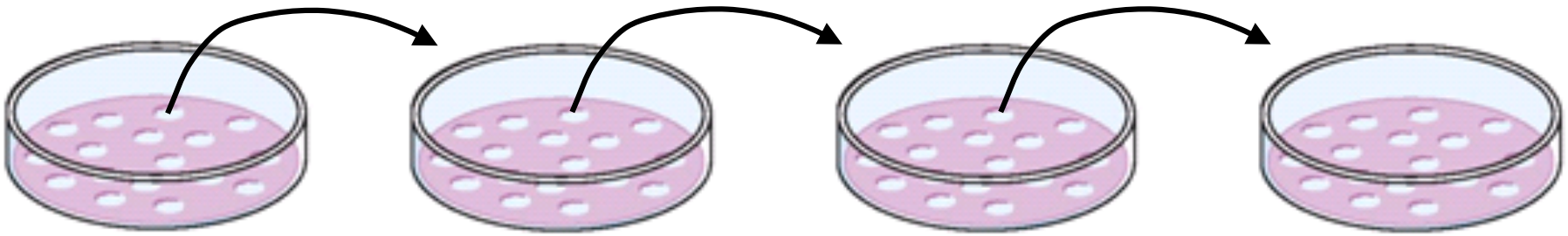
Error threshold

Antiviral *ribavirin* and poliovirus



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



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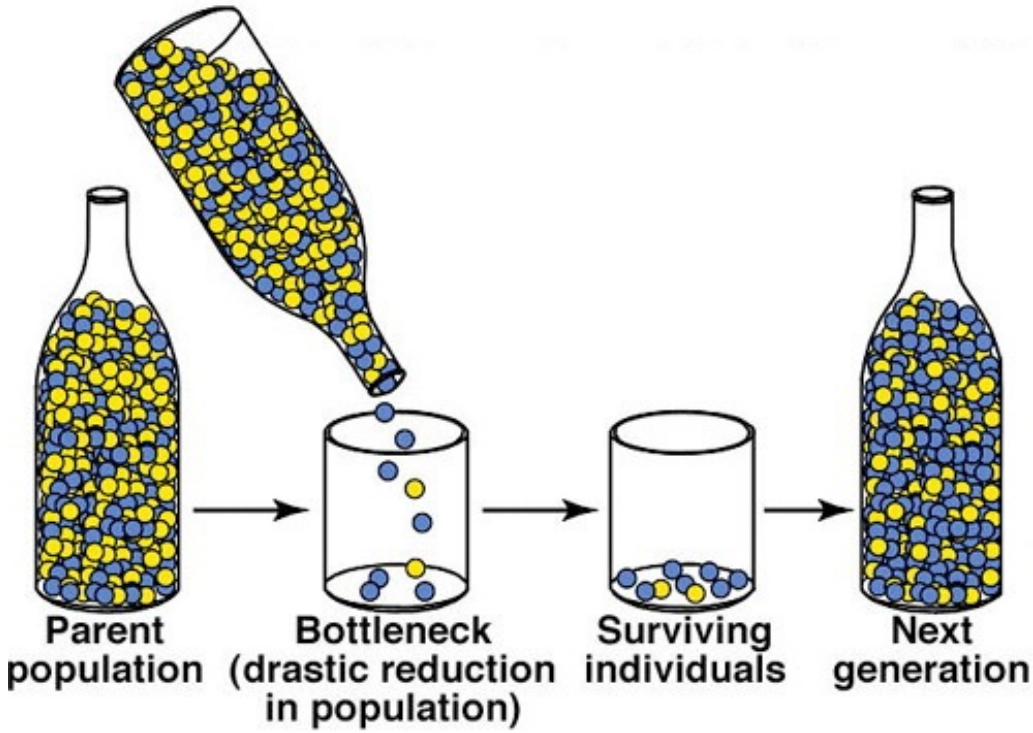
Genetic bottlenecks

- 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?

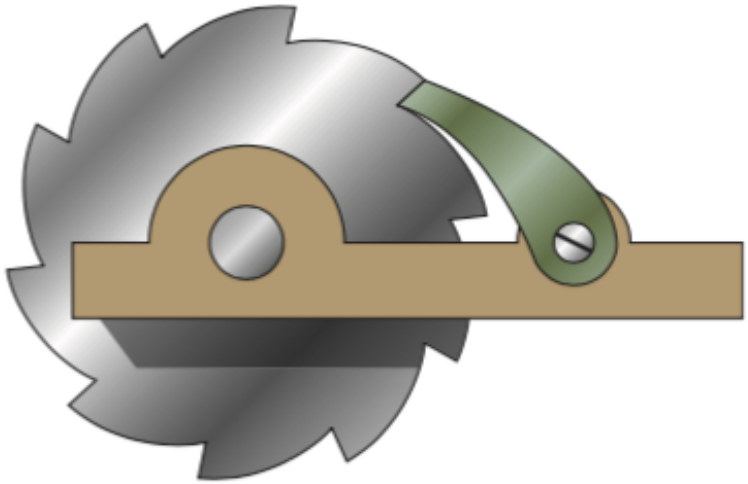
Genetic bottlenecks

- 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



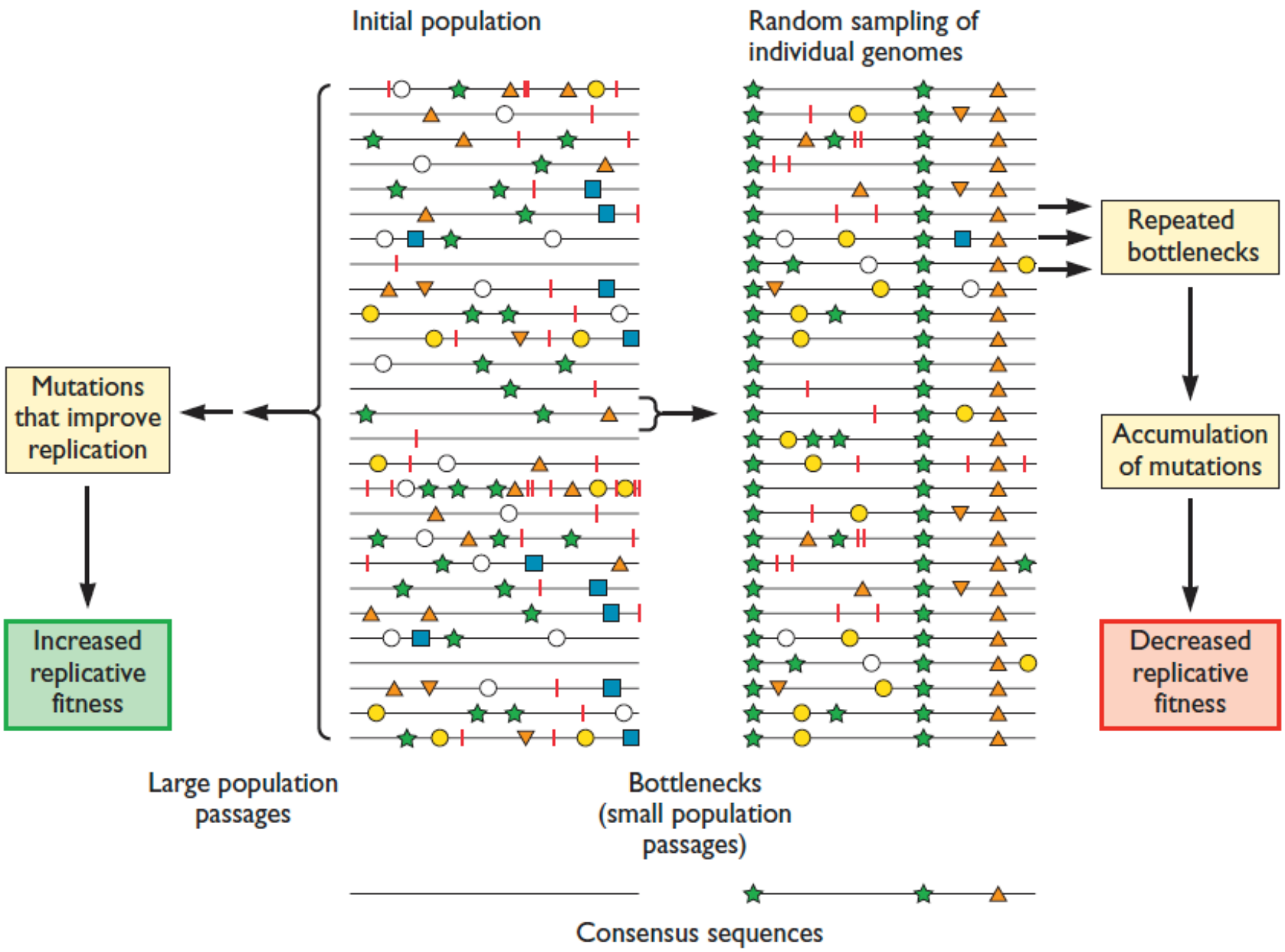
Genetic bottlenecks

- The answer lies in a phenomenon dubbed **Muller's ratchet**: Small, asexual populations decline in fitness over time if the mutation rate is high
- 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



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



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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

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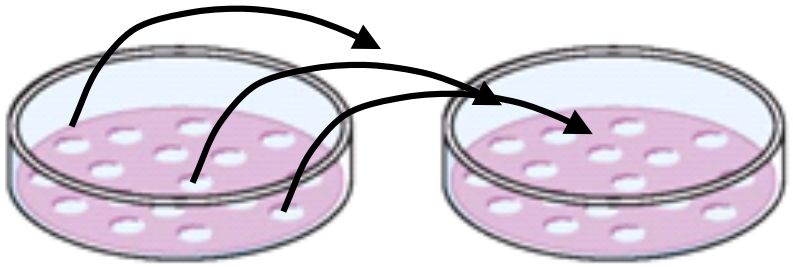
Bottlenecks in the real world?

- 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



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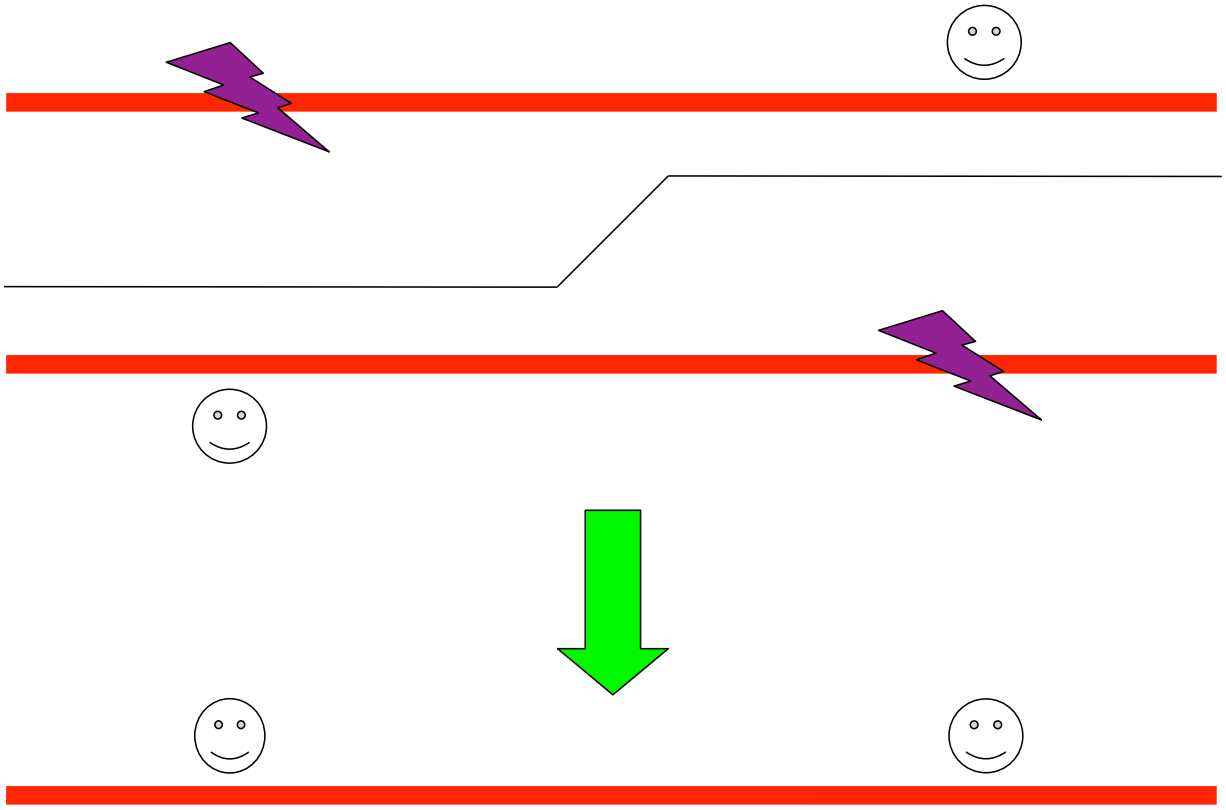
By exchange of genetic information



Sick virus 1

Sick virus 2

Healthy viral recombinant

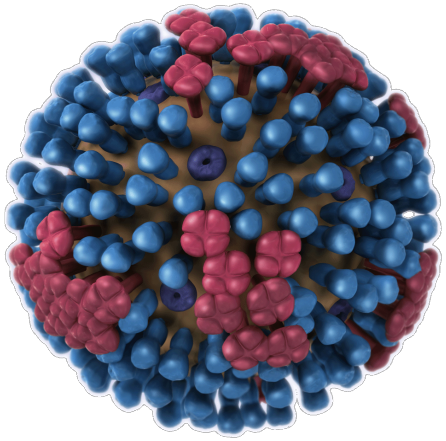
Reassortment

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*

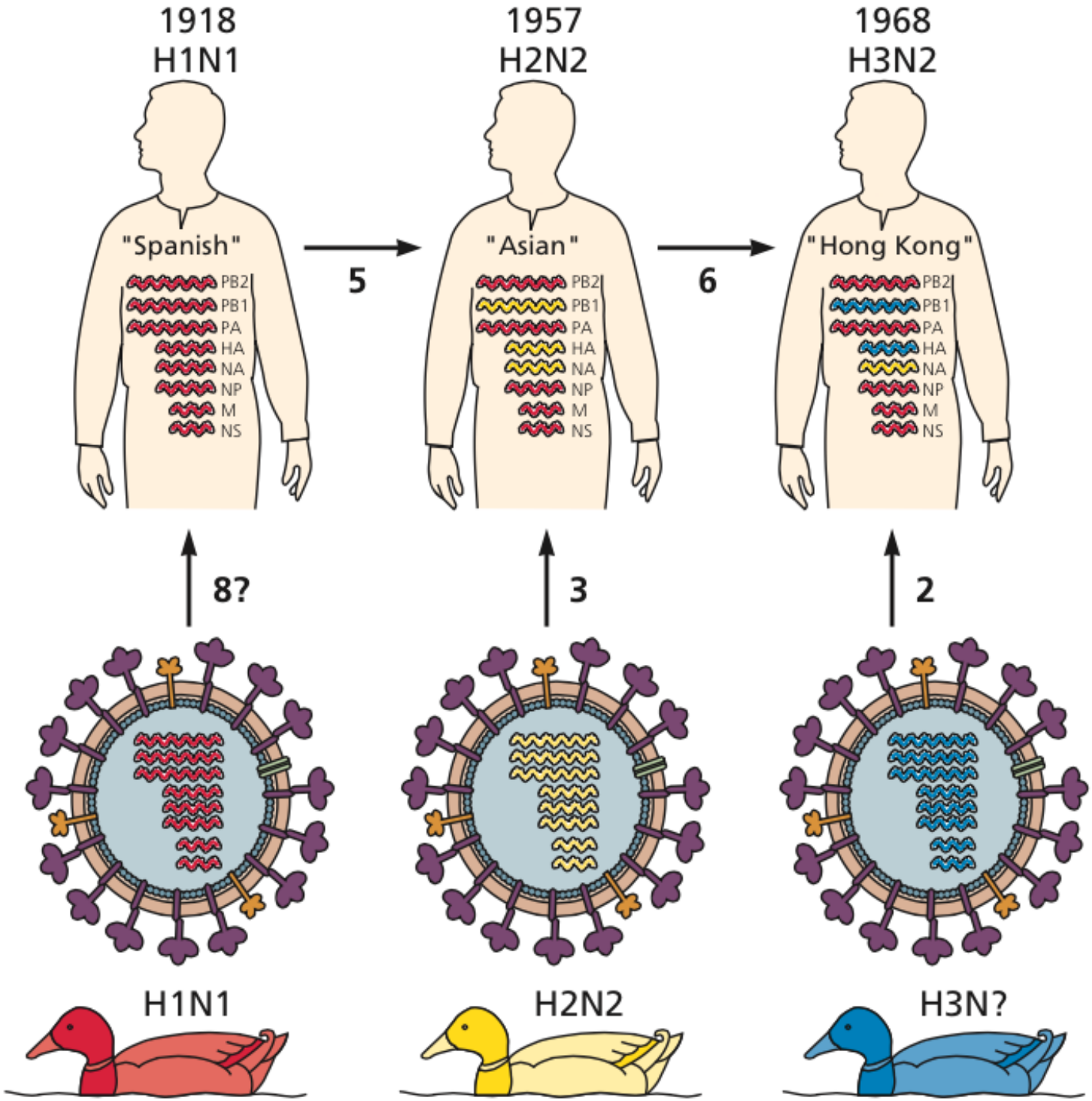
Selection: Genetic shift & drift

- 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 - is relatively rare



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



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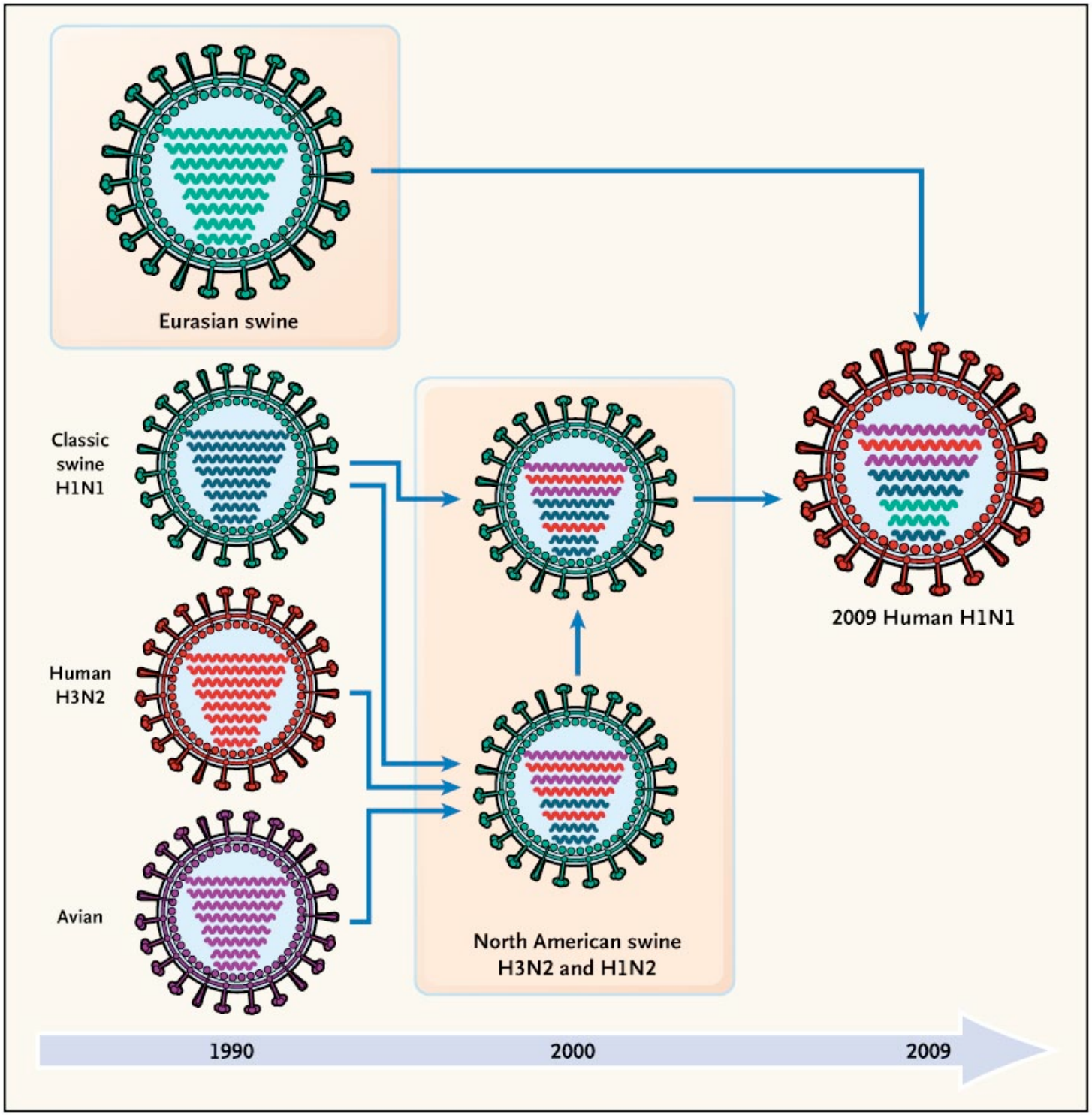
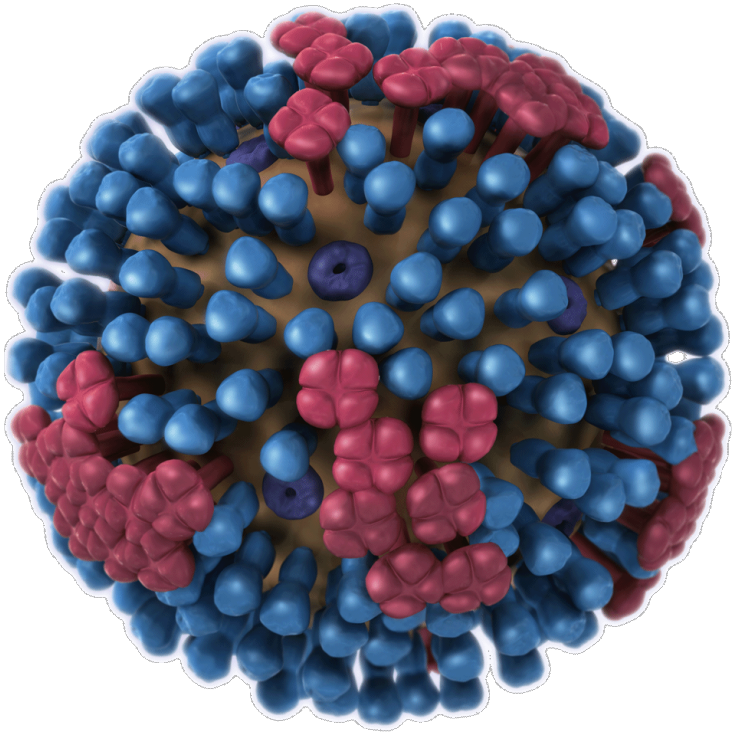
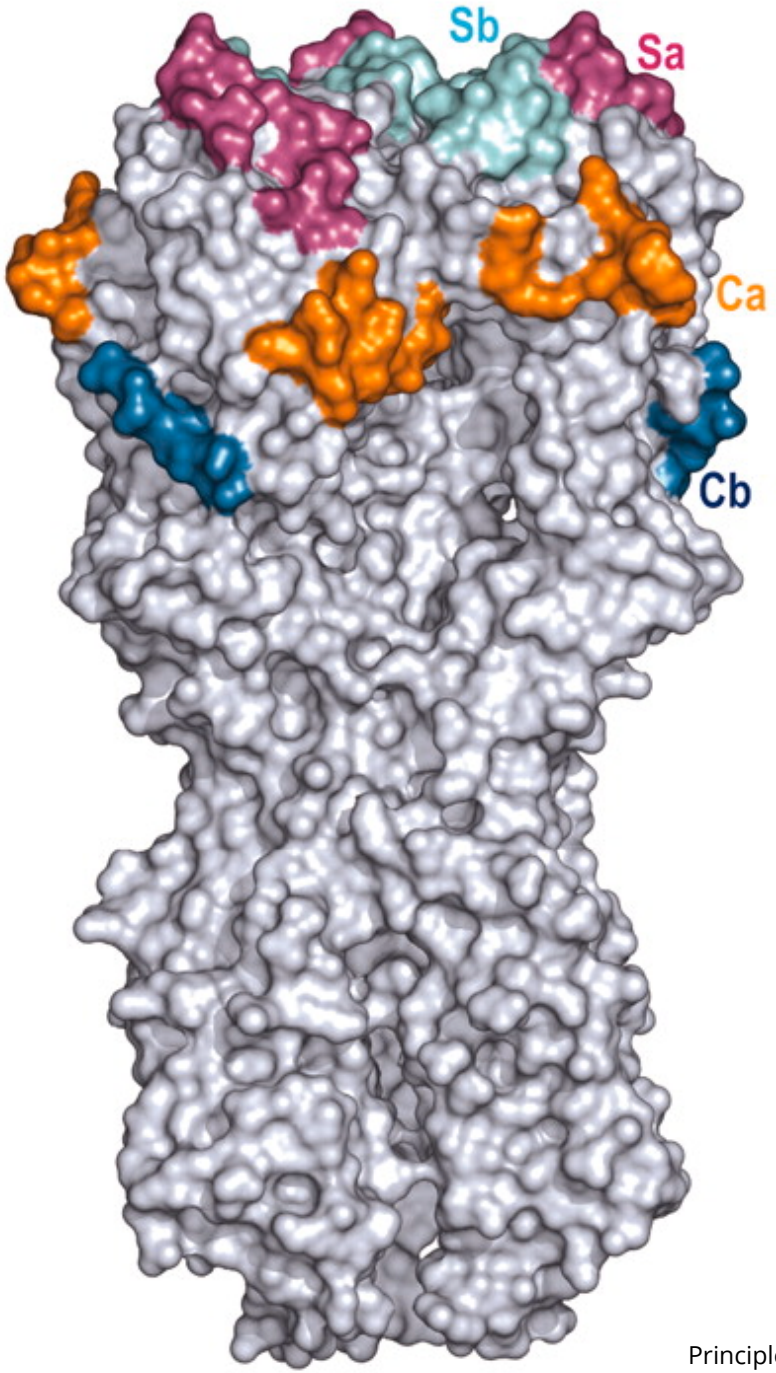

Eurasian swine
Classic swine H1N1
Human H3N2
Avian
North American swine H3N2 and H1N2
2009 Human H1N1
1990
2000
2009

Antigenic drift: Influenza virus





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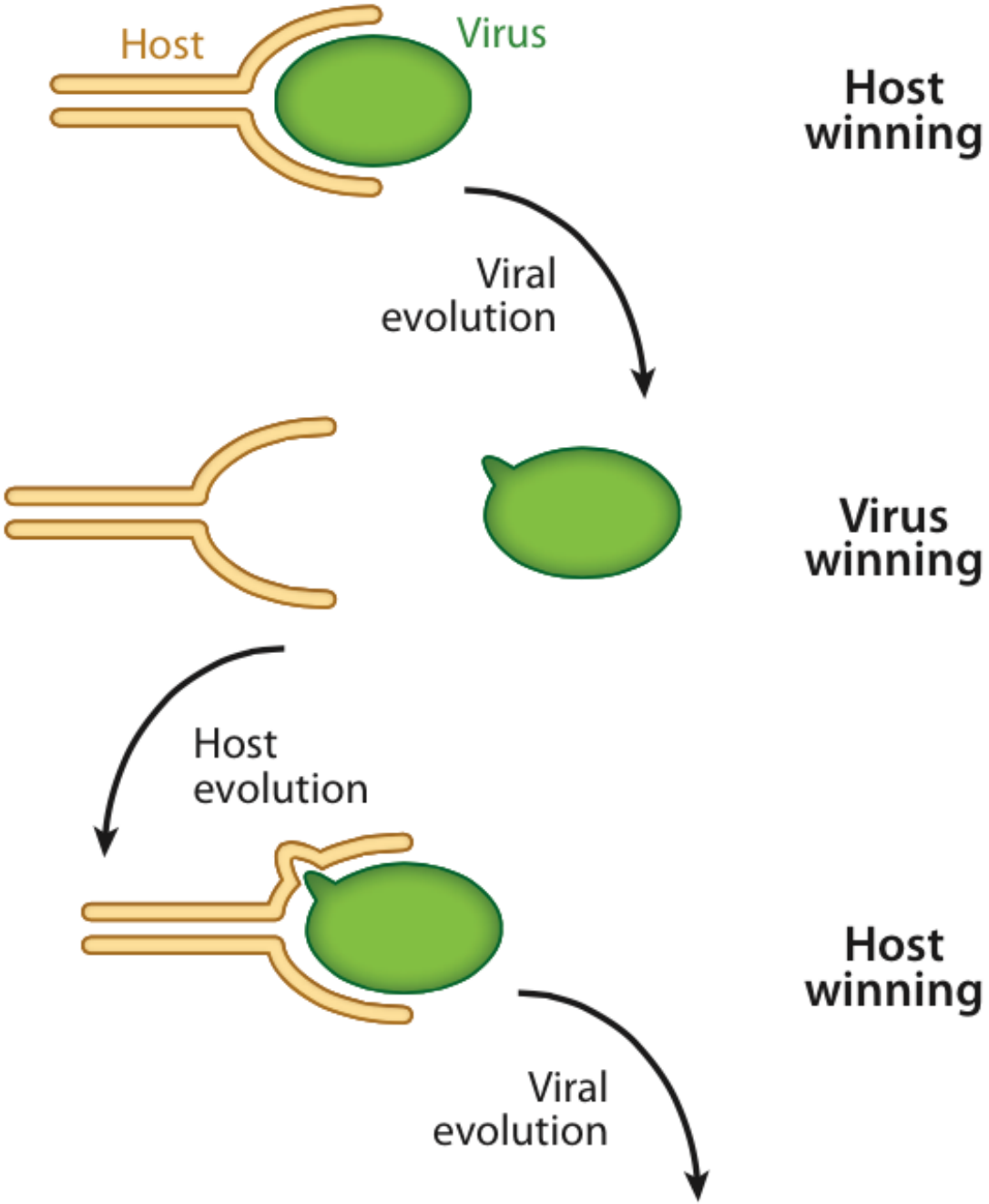
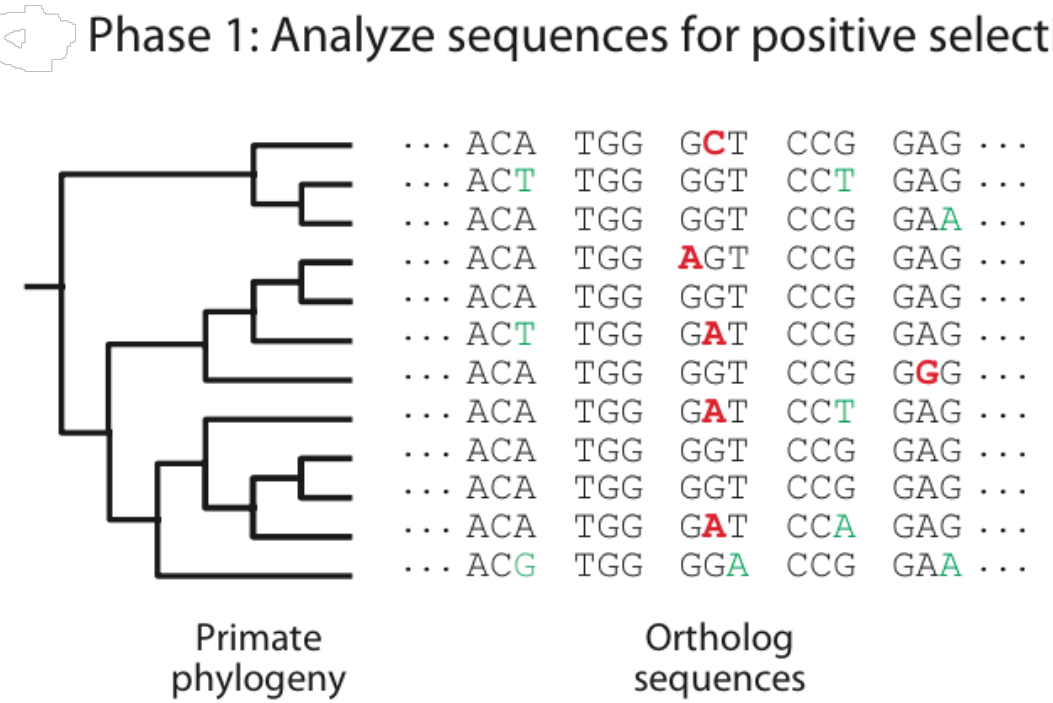
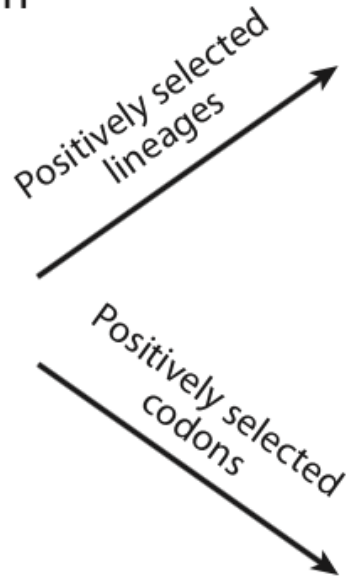
Host-virus arms race

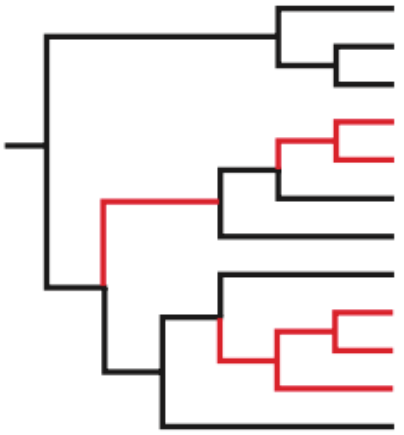


Annu. Rev. Genet. 2012. 46:677–700

Phase 1: Analyze sequences for positive selection







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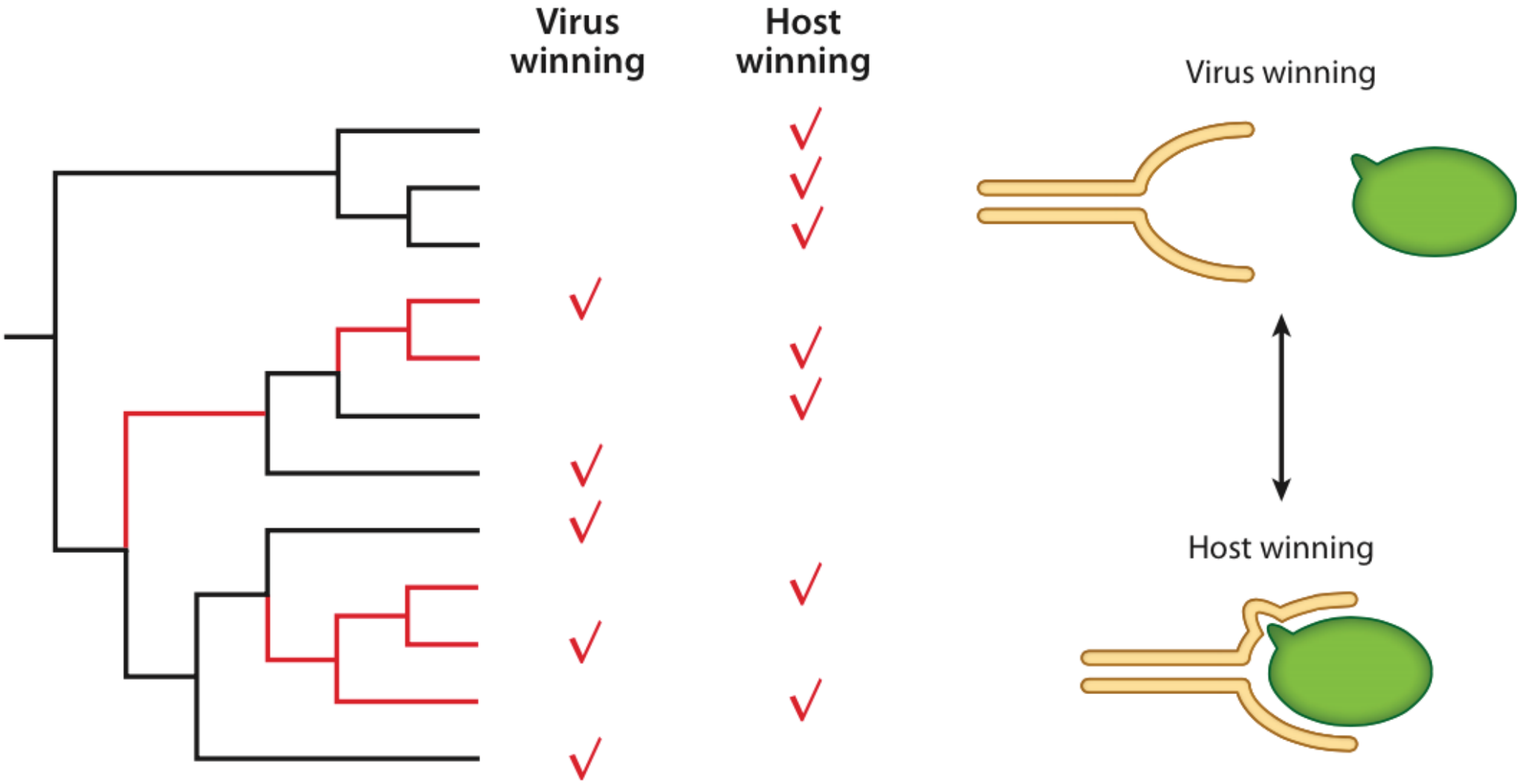
Phase 2: Determine phenotypic consequences of ortholog variation



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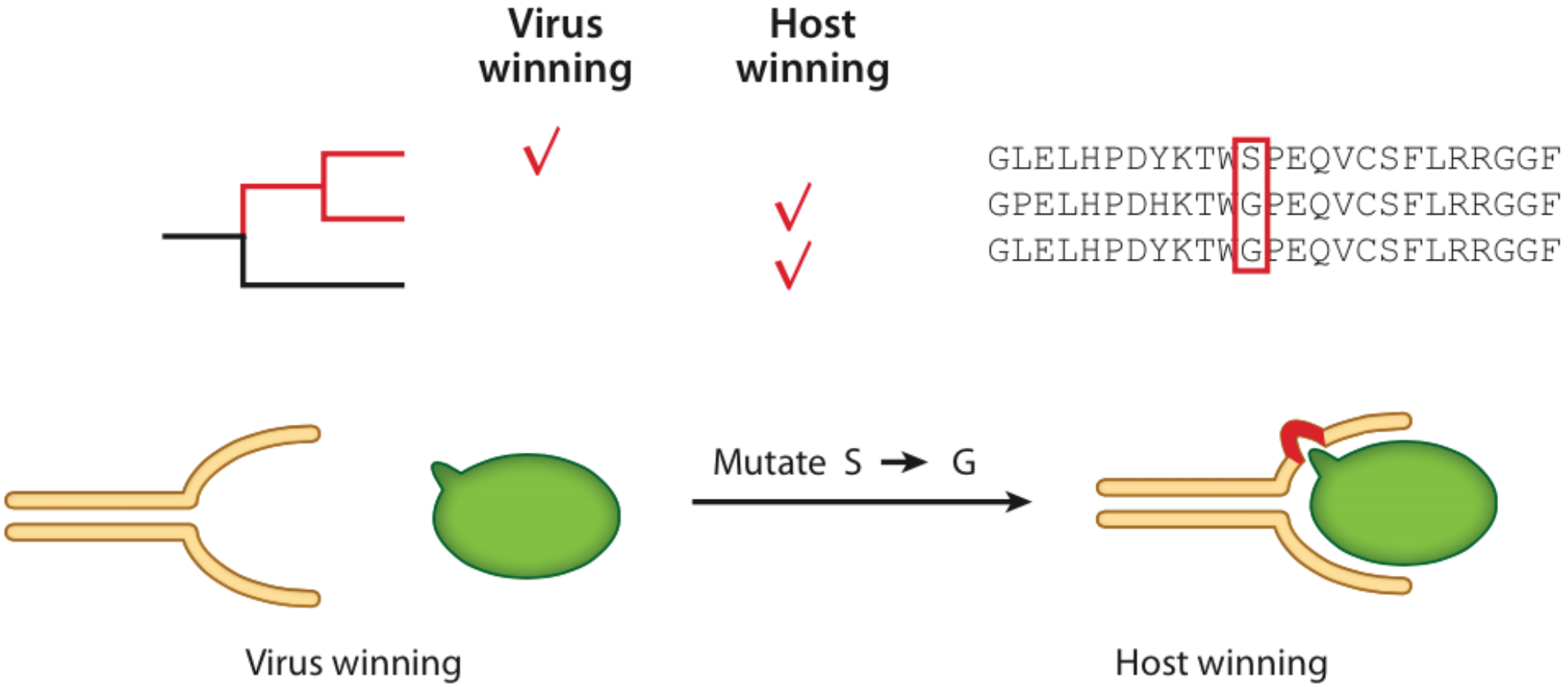
Phase 3: Map positively selected changes onto host-virus interaction



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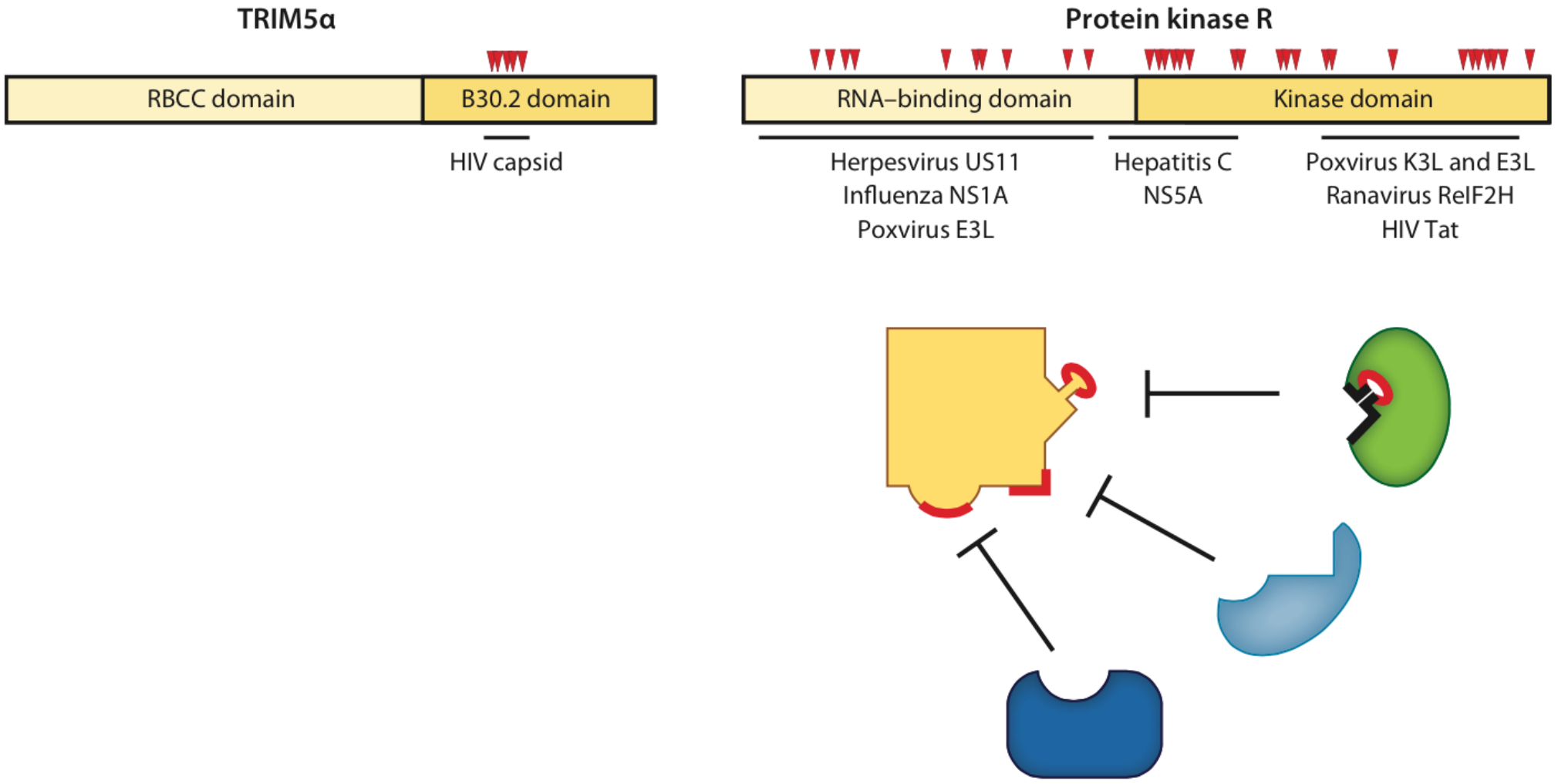
Virus-host conflicts have driven evolution of the immune system



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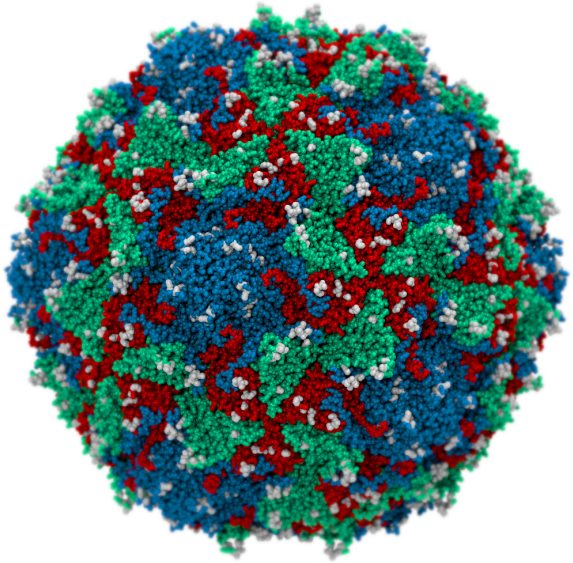
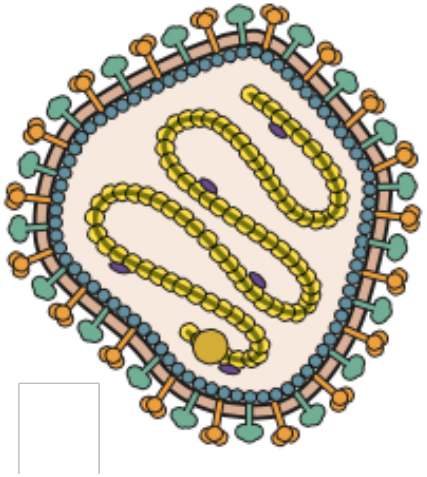
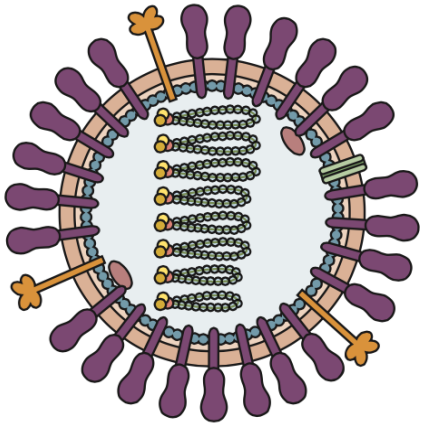
Despite this genome diversity…







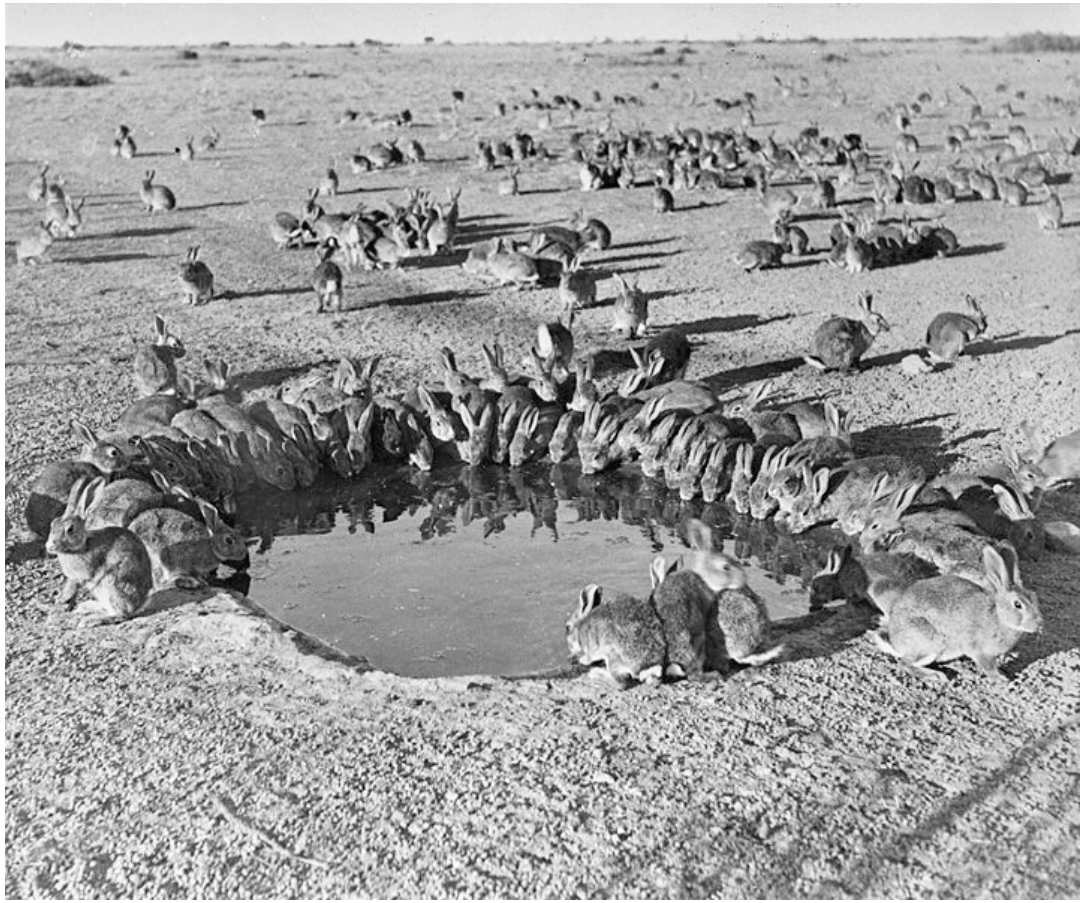
- There are only 3 serotypes of poliovirus, but >150 of rhinoviruses
- One measles serotype, continuous influenza variation
- Why?

Principles of Virology, ASM Press

Selection: Is virulence a positive or negative trait?

- Idea: increased virulence reduces transmissibility because hosts die faster, reducing exposure to uninfected hosts
- Expectation: all viruses evolve to be maximally infectious and avirulent
- But this is not observed - there are many virulent viruses

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

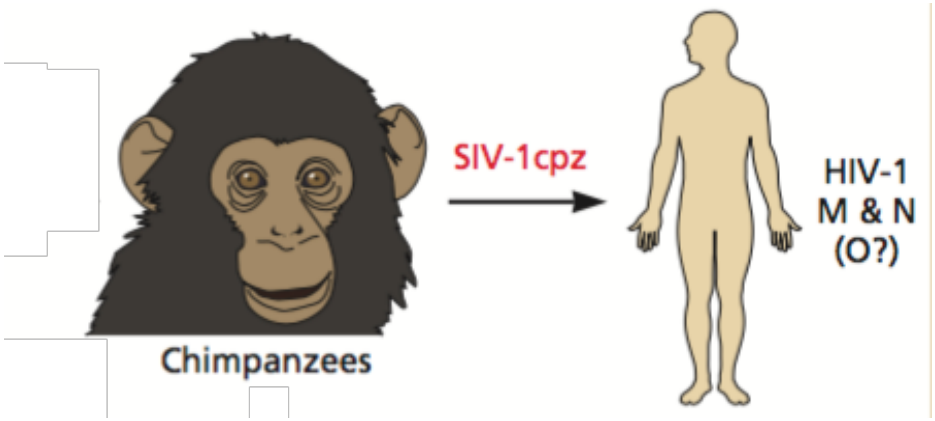
Evolution of virulence

- 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 evolved to kill fewer rabbits and to 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

Evolution of viral virulence in humans?



- Experience with Lassa virus, Ebolavirus, HIV: animal-human virus transfers tend to be virulent
- But viruses from older jumps (measles, poliovirus) are less virulent
- What happened in the meantime?

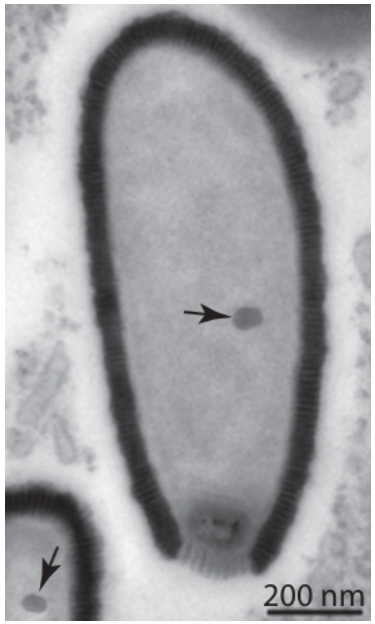
Nevertheless, the press is obsessed with increased viral virulence

- 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.”

We have no data on the effect of evolution on virulence of human viruses

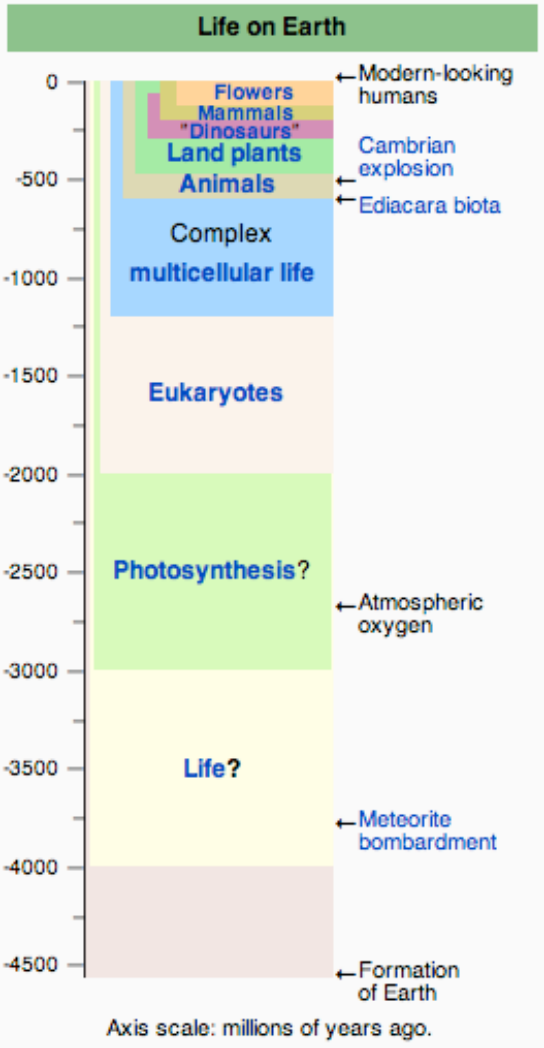
The origin of viruses

Oldest viral stocks:
1918 influenza virus
Pithovirus sibericum
(30,000 y)



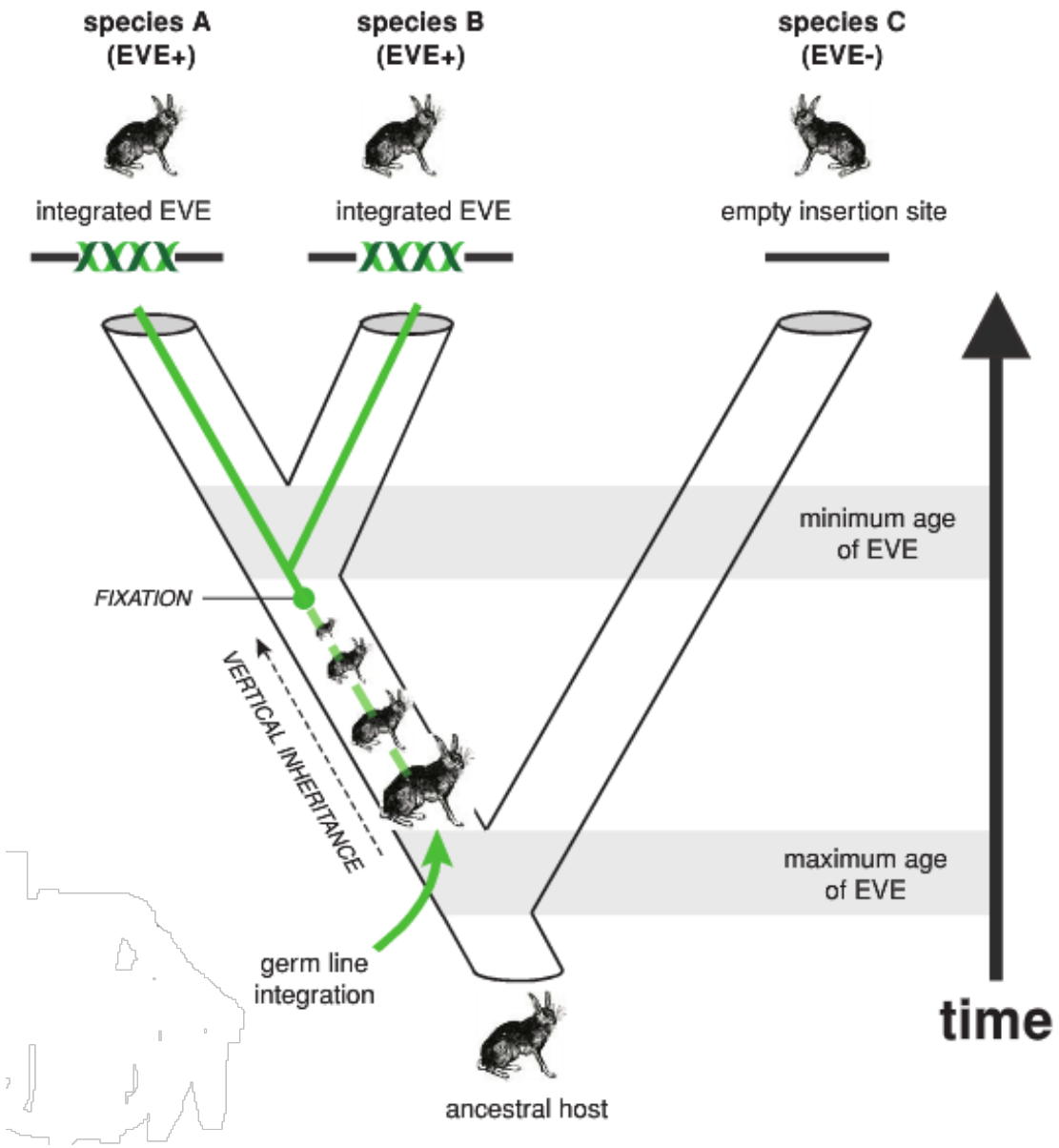
Origins of DNA viruses

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

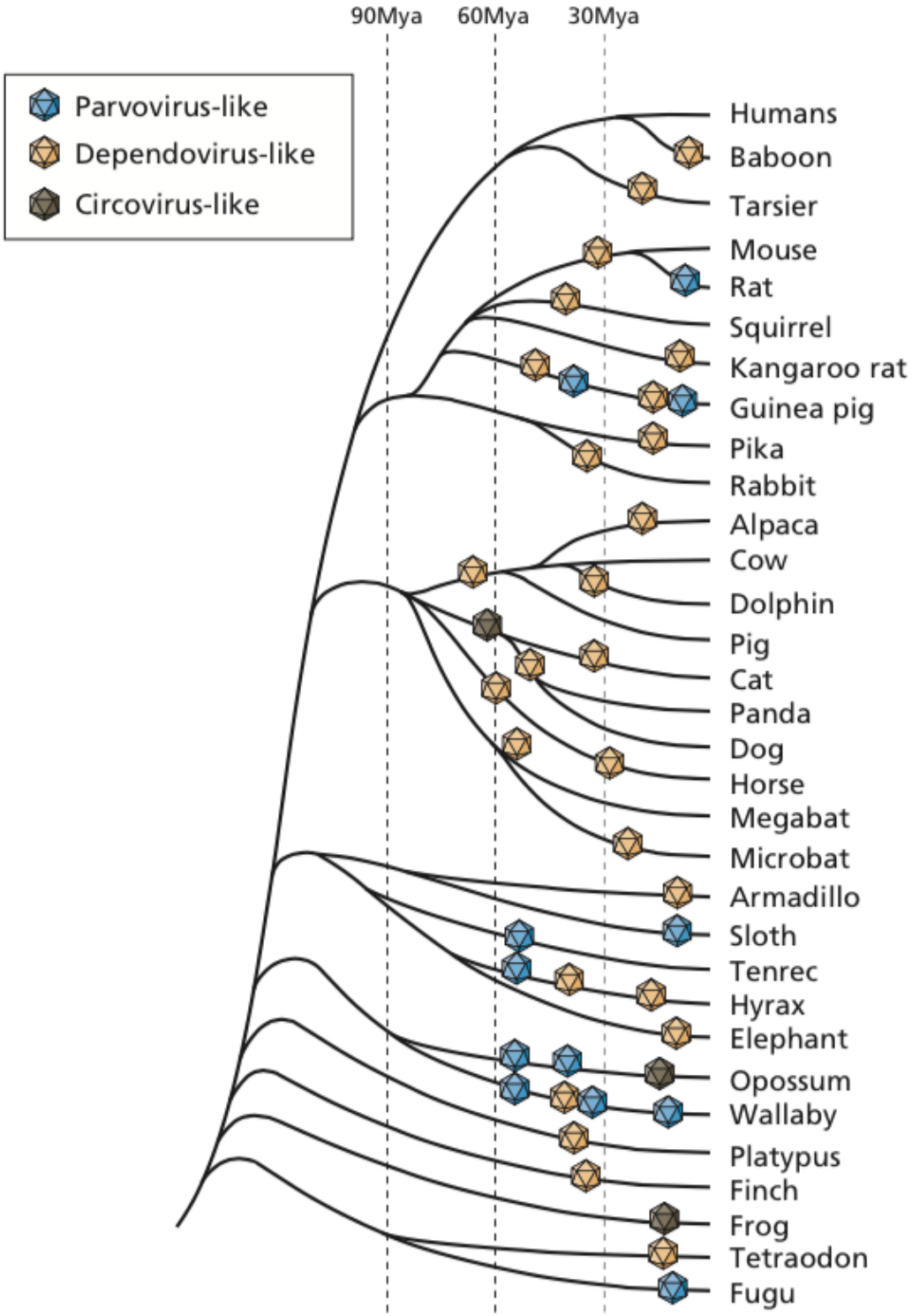


Endogenous viruses - retrovirus and otherwise

Phylogenomics

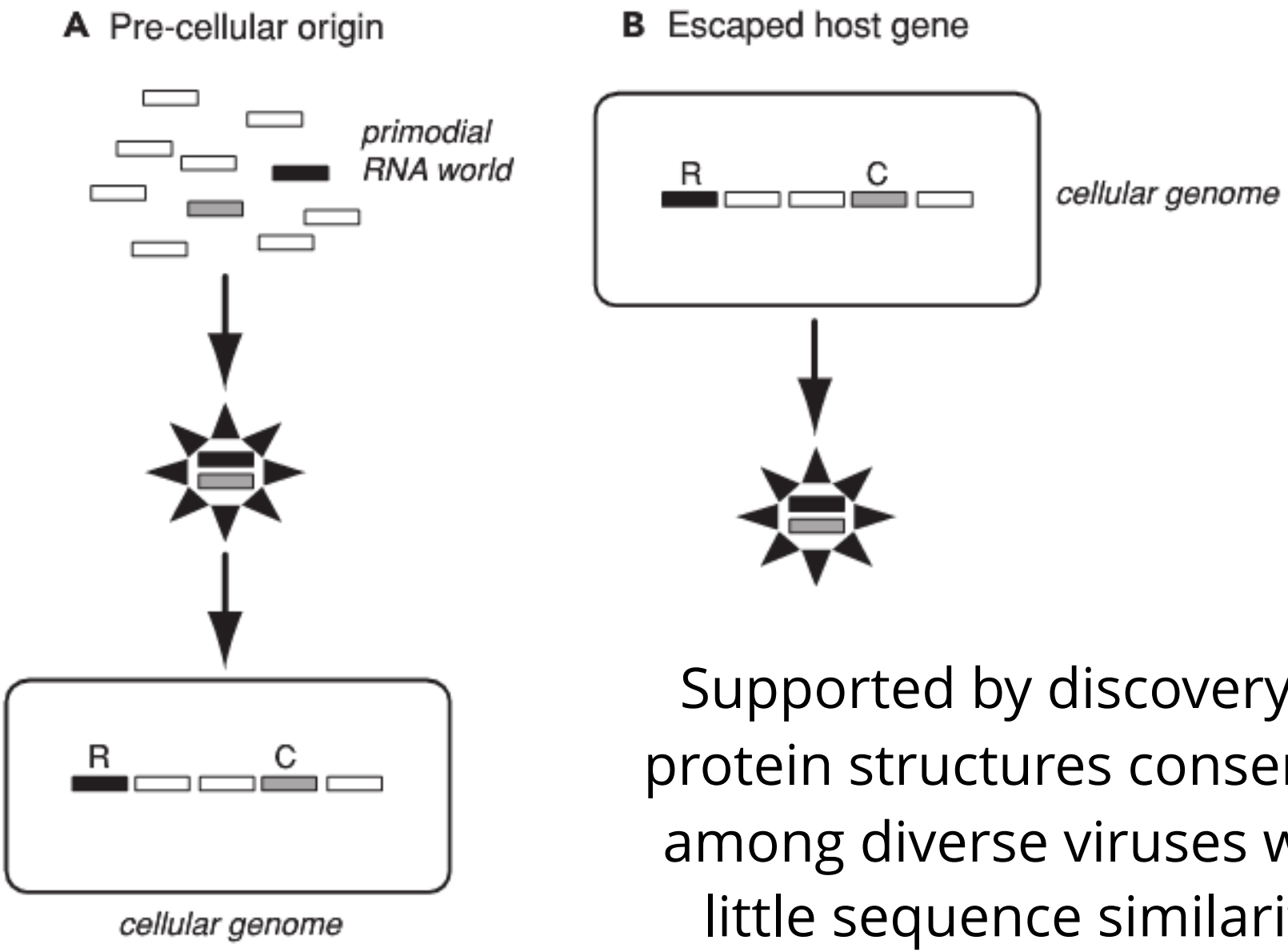


History of ssDNA virus integrations



Principles of Virology, ASM Press

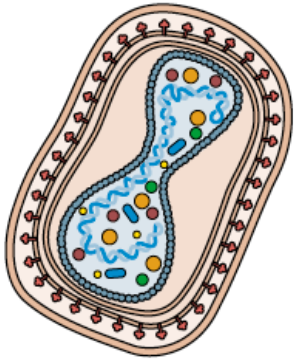
Origins of viruses



Supported by discovery of protein structures conserved among diverse viruses with little sequence similarity

Human viruses

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

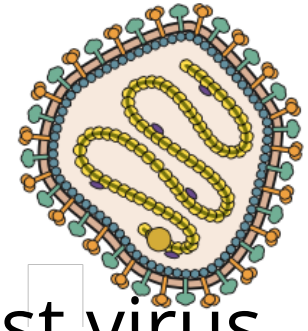


Origins of smallpox virus

- Sequence analysis of 45 isolates: genomes can be organized into 3 clades, which cluster geographically (West Africa, South America, Asia)
- Gene content is constant; lack of diversity indicates recent introduction into humans
- Perhaps human smallpox virus arose after a zoonotic infection from infected gerbils

Principles of Virology, ASM Press

Origins of measles virus



- Measles virus is closely related to rinderpest virus, a bovine pathogen
- Probably evolved from an ancestral rinderpest virus when humans first began to domesticate cattle
- Established in the Middle East ~5,000 years ago, when human populations began to congregate in cities
- Spread around the world by colonization and migration, reaching Americas in 16th century and destroying native Americans (!)

Principles of Virology, ASM Press

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
- Deletions, recombination, and reassortment increases the numbers
- ~4^{135} atoms in the visible universe

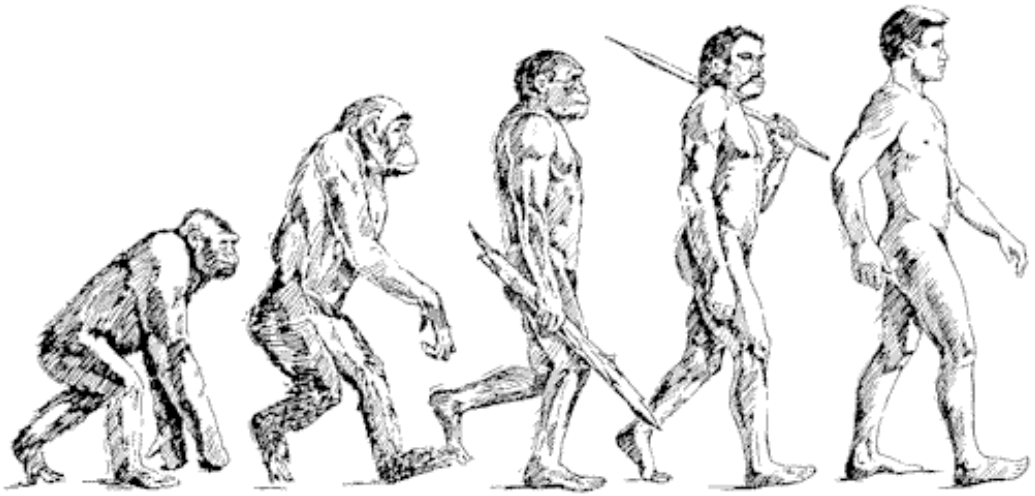
The fundamental properties of viruses constrain and drive evolution

- 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?

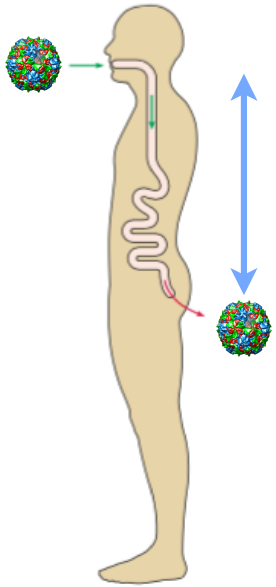
Constraining viral evolution

- Extreme alterations in viral consensus genome do not survive selection
- The viral genome is one constraint
  - *DNA cannot become RNA, or vice versa*
  - *Replication strategy - cannot change; consider interaction with host proteins*
- 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, suffer the same fate as one that does not replicate efficiently enough*

Food for thought



8 million years



5 days

Imagine what a virus can do with 8 million years

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