100 YEARS POLIOVIRUS
FROM DISCOVERY TO ERADICATION
UNDER THE AUSPICES OF THE AUSTRIAN ACADEMY OF SCIENCES AND THE MEDICAL UNIVERSITY OF VIENNA
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Stability, instability, and evolution of the poliovirus genome

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Our understanding of genetics and evolution of RNA-viruses are largely based on the knowledge derived from studies on poliovirus – *again a pathfinder*

What are the major lessons?

Prehistory (~50 yr after the virus discovery)

Poliovirus discovery (*Landsteiner & Popper, 1909*)

First evidence for poliovirus variability:

- Serotypes (*Burnet & Macnamara, 1931*).
- Selection: host range - adaptation to rodents (*Armstrong, 1939*).
- Selection: virulence & attenuation (*Koprowski, Jervis, & Norton, 1952*).

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Methodological breakthroughs:

- Cultivation in non-neural tissues (*Enders, Weller, & Robbins, 1949*).
- Cultivation in cell monolayers; the term CPE (*Robbins, Enders, & Weller, 1950*).
- Plaque technique (*Dulbecco 1952; Dulbecco& Vogt, 1954*).

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RNA genome:

- Structural component (*Schwerdt & Schaffer, 1955*).
- Infectivity (*Colter et al.,1957*).
- Single single-stranded molecule (*Holland et al., 1960*).

Prehistory (~50 yr after the virus discovery)

Discovery of phenotypic markers:

- Temperature-sensitivity (*Dubes & Wenner, 1957; Lwoff & Lwoff, 1959*).
- Resistance to inhibitors (*Melnick, Crowther, & Barrera-Oro, 1961*).

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Mutagenesis:

- In vivo - incubation with proflavine (*Dulbecco & Vogt, 1958*).
- In vitro – chemical treatment of viral RNA (*Boeye,1959*)

The major achievements of the prehistory:

- **development of vaccines, IPV (*Salk*) and OPV (*Sabin*)**
- **creation of a model system for molecular virology**

Fundamental problems pertinent to these achievements:

- **stability of the viral genome**
- **its variability &**
- **evolution**

Further progress in understanding of poliovirus genetics heavily depended on two crucial breakthroughs:

- **Determination of primary structure of the viral genome**

(Racaniello and Baltimore, 1981; Kitamura et al., 1981)

and

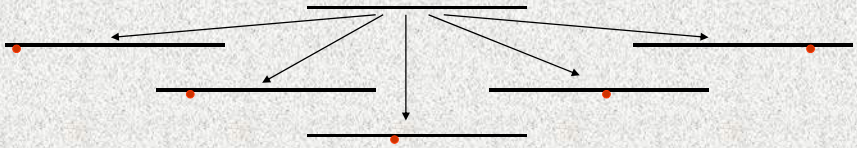
- **Demonstration of infectivity of the viral cDNA and introduction of reverse genetics**

(Racaniello and Baltimore, 1981)

What are the key regularities?

Inborn infidelity:

Due to the RdRP infidelity, each synthesized RNA molecule acquires, on average, a mutation



Life at the edge of mutational catastrophe

A few-fold increase in the error frequency → extinction of a population

Is it good or bad for the virus?

Because of such grammatical negligence any poliovirus population is *heterogeneous* (a quasi-species)

Heterogeneity confers a high adaptability

It explains extreme shortage of antiviral drugs

Thus, RNA viruses are happy not only despite, but also because of, their infidelity

Any poliovirus population is not a herd of uniform entities but rather a dynamic society composed of competing and cooperating members

The cooperation is very important:

- A single mutation may ***increase*** the fidelity of polio RdRP
(*Pfeiffer & Kirkegaard, 2003*)
- The progeny of this mutant is ***more homogenous*** but **LESS FIT**
(*Pfeiffer & Kirkegaard, 2005*)
- ***More homogeneous*** populations are ***more attenuated***
(*Vignuzzi et al., 2006*)

A decrease in fitness appears to be due to the lack of cooperation within the population

The possibility of cooperation between simple animal RNA viruses

(= *complementation*)

was originally discovered just in poliovirus

(Cords & Holland,, 1964; Agol & Shirman, 1964; Wecker & Lederhilger,, 1964)

Nevertheless, in view of such genome instability,

how to maintain the identity?

how to survive?

Upon natural circulation, polioviruses retain their identity and fitness.
Hence, the virus knows the answers

What are they?

Two remarkable properties of the viral genome:

- ***Robustness*** **–**

  retention of fitness or at least viability in spite of a variety of changes

- ***Reparability*** **–**

  capacity to restore impaired functions

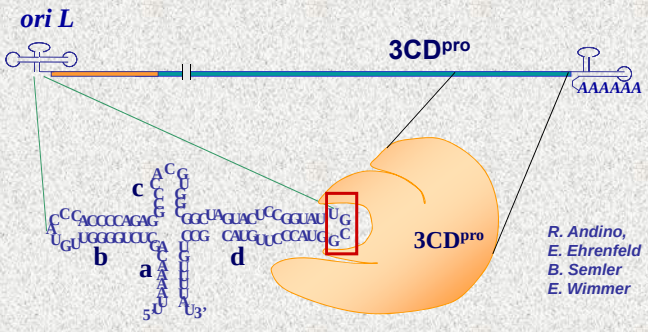
Robustness

- **Many point mutations are neutral or nearly so**

  In the coding region, they are often synonymous

- **Combinations of point mutations may also be neutral or nearly so**

  Even in the conserved cis-elements of the genome



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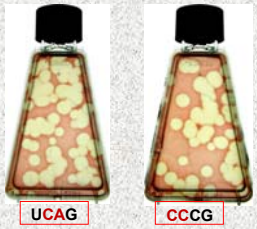
Conserved wt



Changed tetraloop *d* sequences

(Bakhmutov et al., unpubl.)

Reparability

Fitness-decreasing mutations tend to be phenotypically repaired

This reparability is due to a combination of

the infidelity of RNA replication, which creates variability

and

purifying (negative) selection, which removes less fit variants

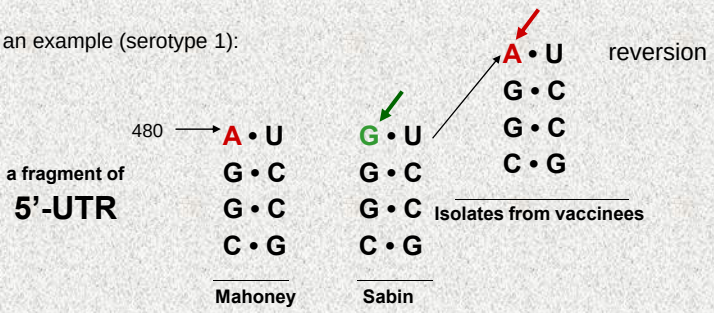
Versatility of reparation machinery

Restoration of the fitness may occur *via*

- ***Reversions***

The attenuating mutations of the Sabin strains tend to revert

Just an example (serotype 1):



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Restoration of the fitness may occur *via*

- ***Reversions***
- ***Pseudoreversions***

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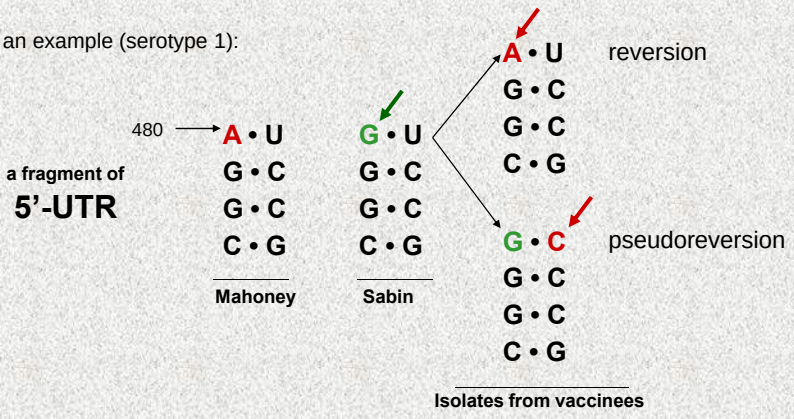
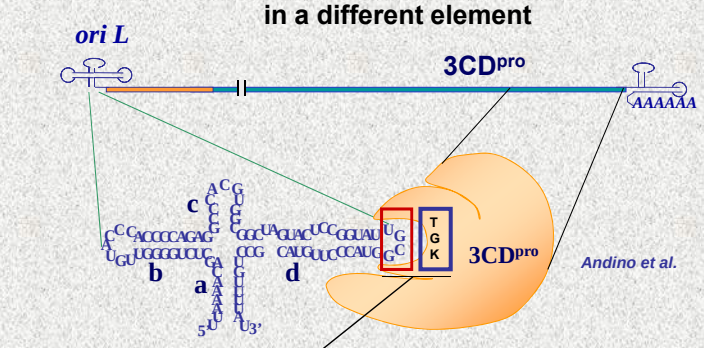
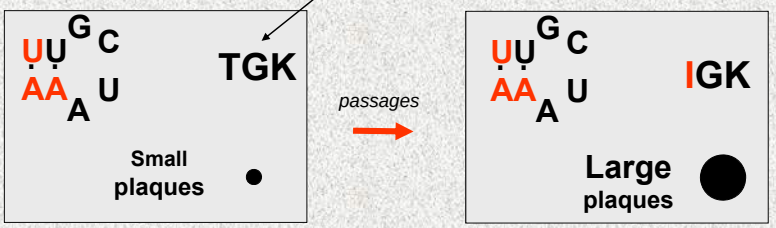


Versatility of reparation machinery

Fitness restoration may be due to a mutation(s) at a distant site and in a different element



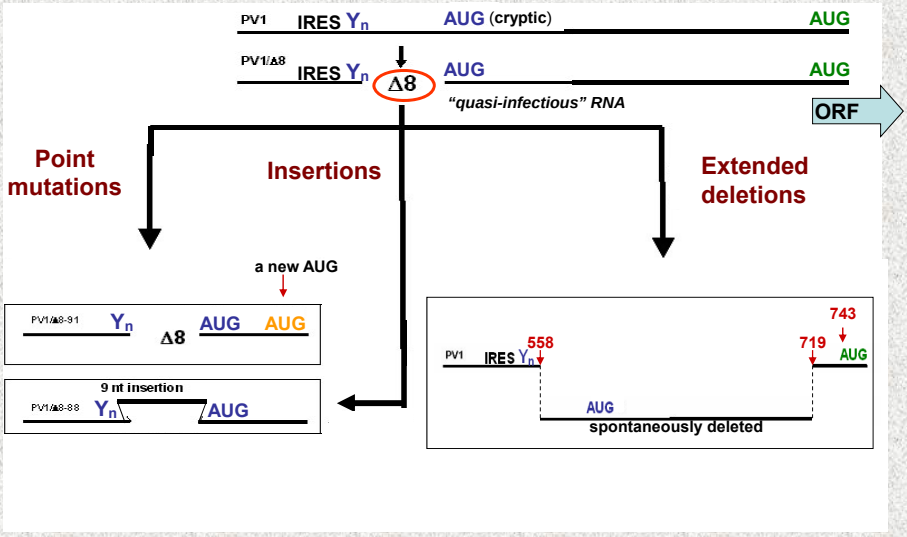


An easy way to change

(Bakhmutov et al., unpubl.)

Versatility of reparation machinery

Fitness may be restored even after severe genome damages



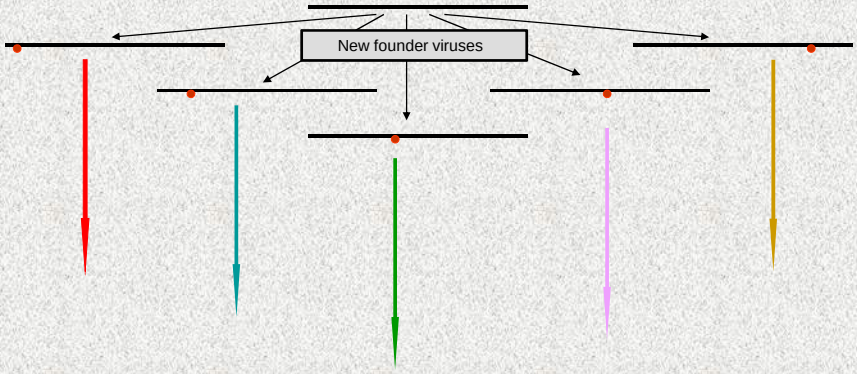
Redesign of a new control element from "spare parts"

(Pilipenko et al. Cell 1992
Gmyl et al. J. Virol. 1993)

Being robust and repairable, even well fit (wt) polioviruses are evolving

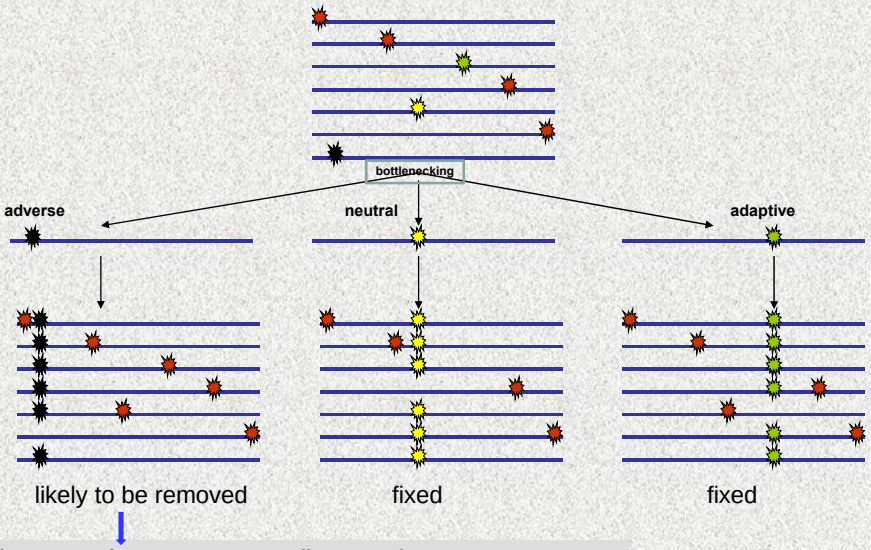
Evolutionary factors:

•**Bottlenecking** (picking up accidental mutations from a heterogeneous population)
Likely to occur upon natural transmission.

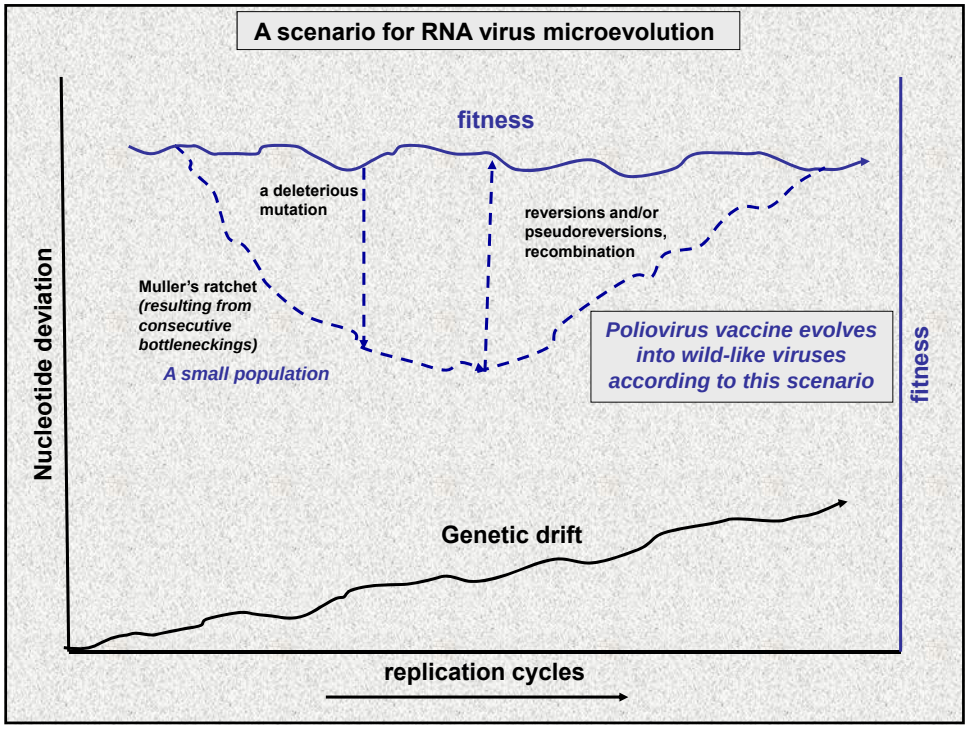


Evolutionary factors:

•**Environmental changes** (re-evaluating adaptive significance of mutations)



Adverse mutations are not necessarily removed.
Consecutive bottlenecking may result in a significant fitness decrease,
even to extinction of a population –
the Muller's ratchet

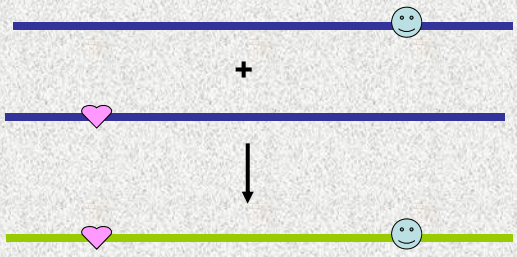


In addition to point mutations, there is another genetic process responsible for viral variability and evolution -

recombination

Intermolecular RNA recombination has been discovered and most thoroughly studied by using just the poliovirus model

- The first genetic evidence (*Ledinko, 1962*)



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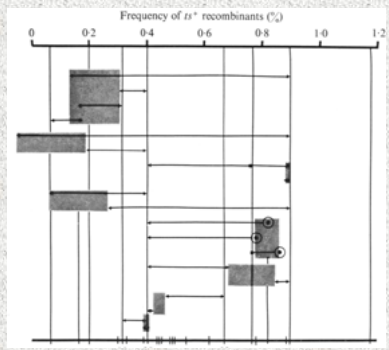

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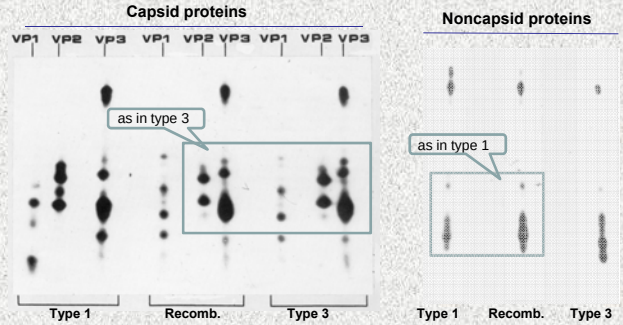
- **First biochemical evidence for RNA recombination** *and* **evidence for intertypic poliovirus recombination (*Romanova et al., 1980*)**



Poliovirus type 3/type 1 recombinant

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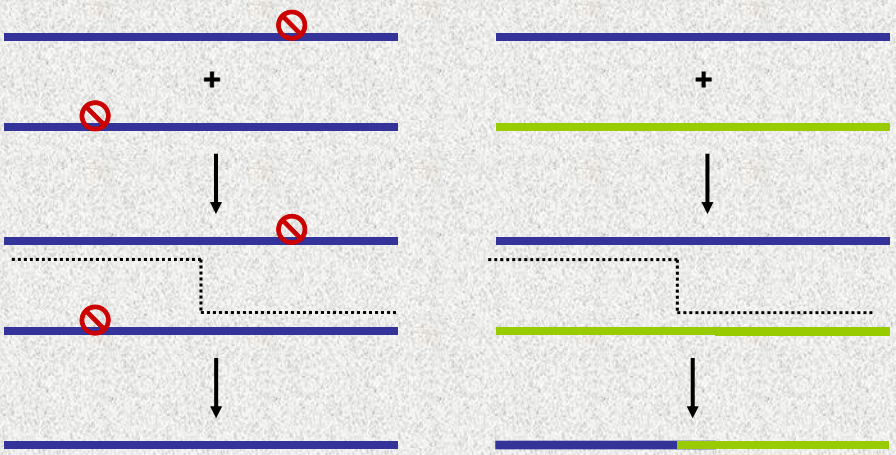
- The additive genetic map of poliovirus ts mutants (*Cooper, 1968, 1975*)
- Evidence for intertypic poliovirus recombination, first biochemical evidence for RNA recombination (*Romanova et al., 1980*)
- **First evidence for natural recombination – in a tOPV recipient (*Kew & Nottay, 1984*)**
- **Frequent isolation of recombinants from patients with vaccine-associated poliomyelitis (*Lipskaya et al., 1991*)**

Dual role of recombination

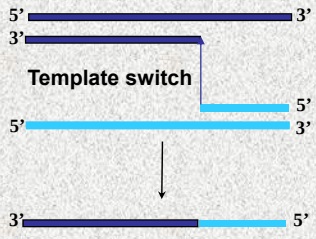


Genome stabilization

Genome change

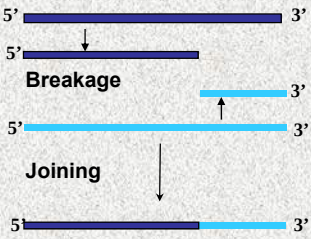
Recombination may occur *via* two fundamentally different mechanisms

replicative



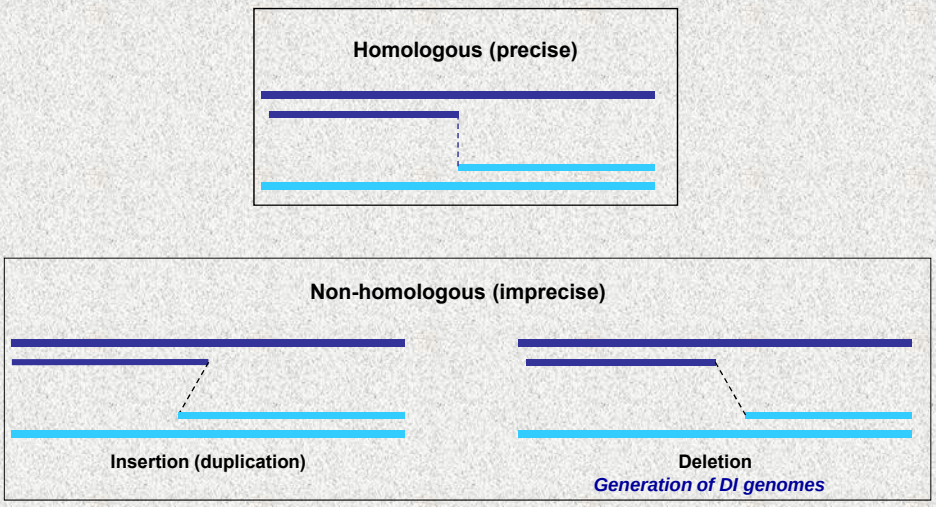
(Cooper, 1974; Kirkegaard & Baltimore, 1986; Romanova et al., 1986)

nonreplicative



(Gmyl et al., 1999; 2003)

Recombination may be homologous or non-homologous



Generation of DI genomes

Recombination may or may not change the fitness

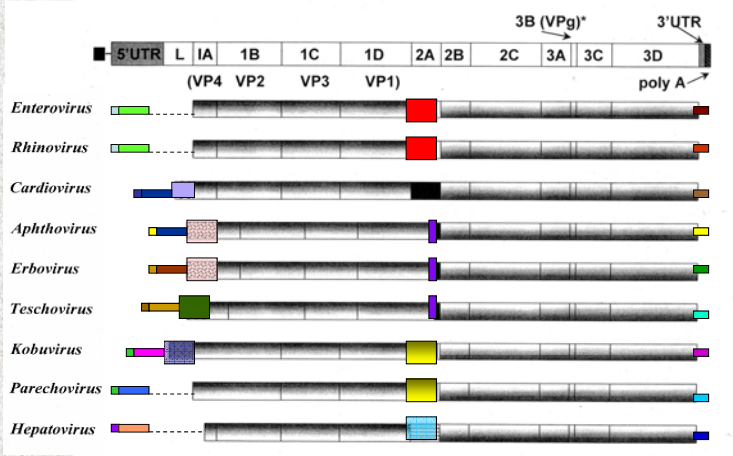
neutral change

adaptive change

Recombination is a major factor of macroevolution

(formation of novel taxa)

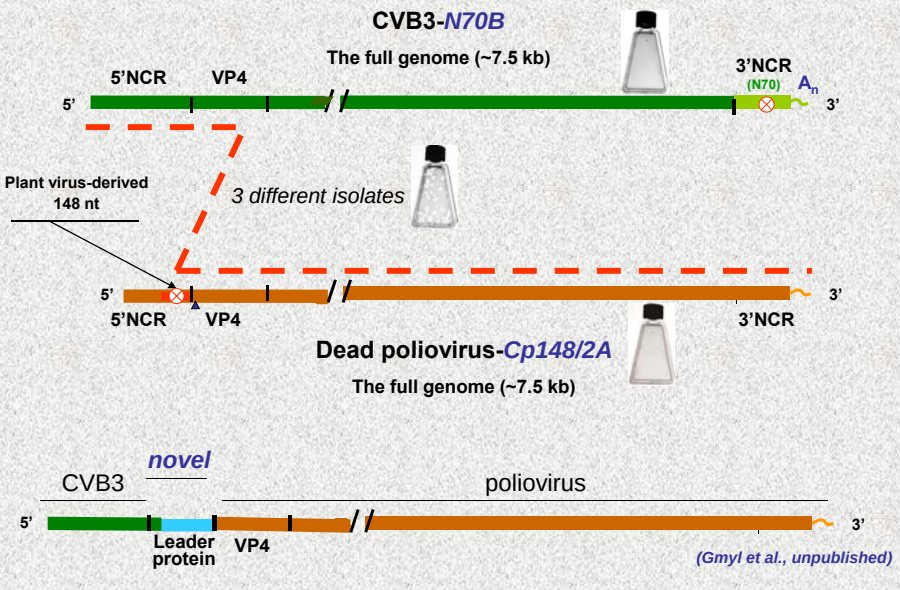
Genome organization of different picornavirus genera



Several structurally different types of IRES
Several structurally different types of 5'-NTR
Several structurally different types of 3'-NTR
Structurally and functionally different variants of L protein (or its absence)
Structurally and functionally different variants of 2A protein

Recombination may result in the "non-coding → coding" sequence conversion

Interspecies recombination



(Gmyl et al., unpublished)

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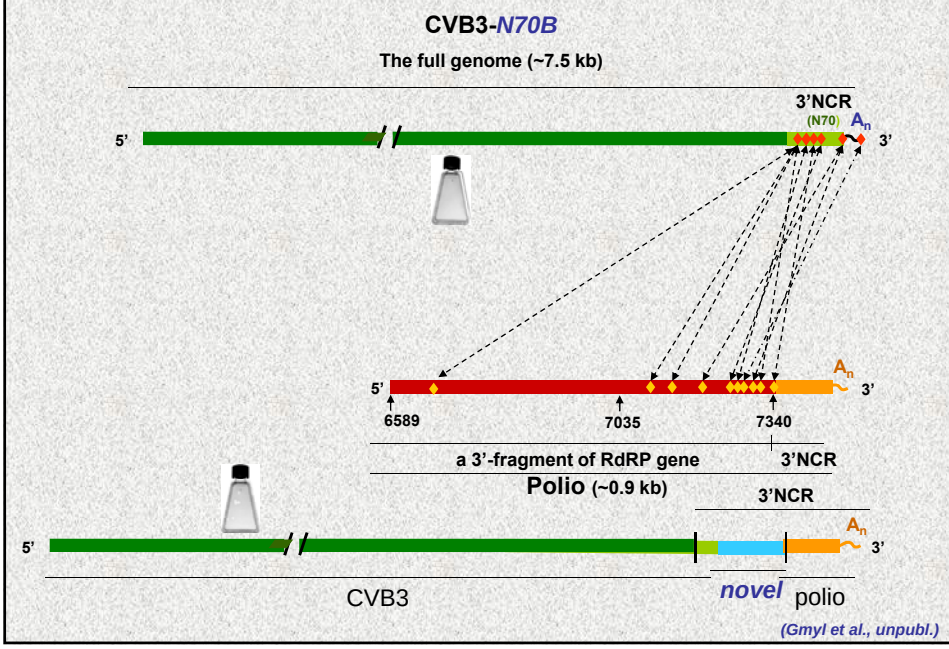


This is <u>the first</u> experimental demonstration of <u>interspecies</u> RNA recombination in picornaviruses. Why the first?

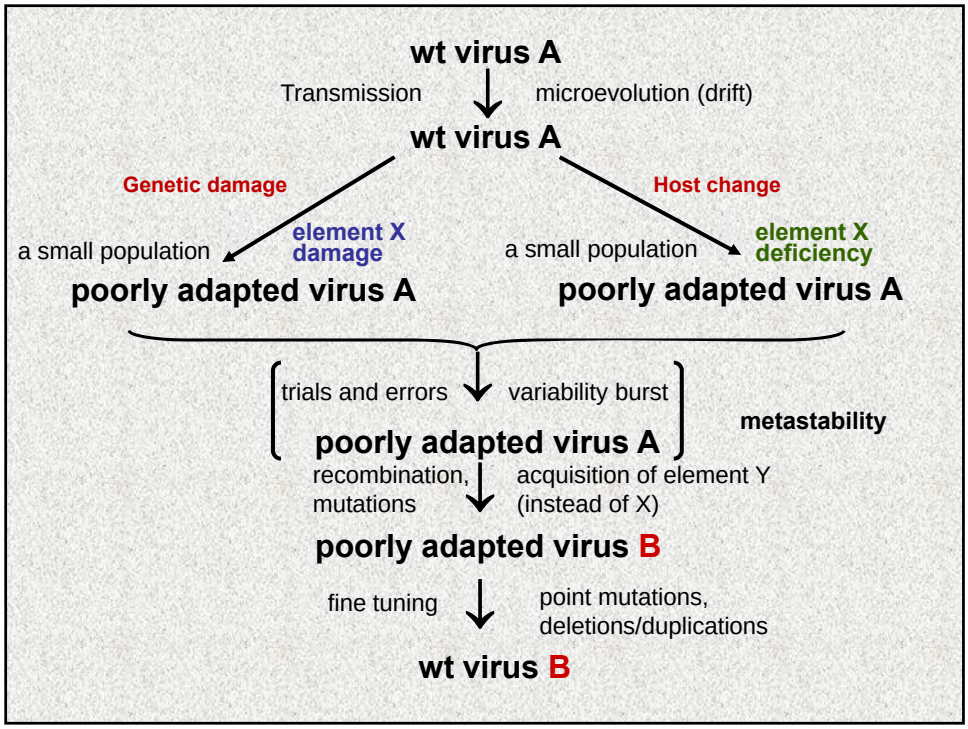
All such (and many other) interspecies recombinants are likely formed upon mixed infection with wild type CVB and poliovirus but, being an extreme minority, remain undetected.

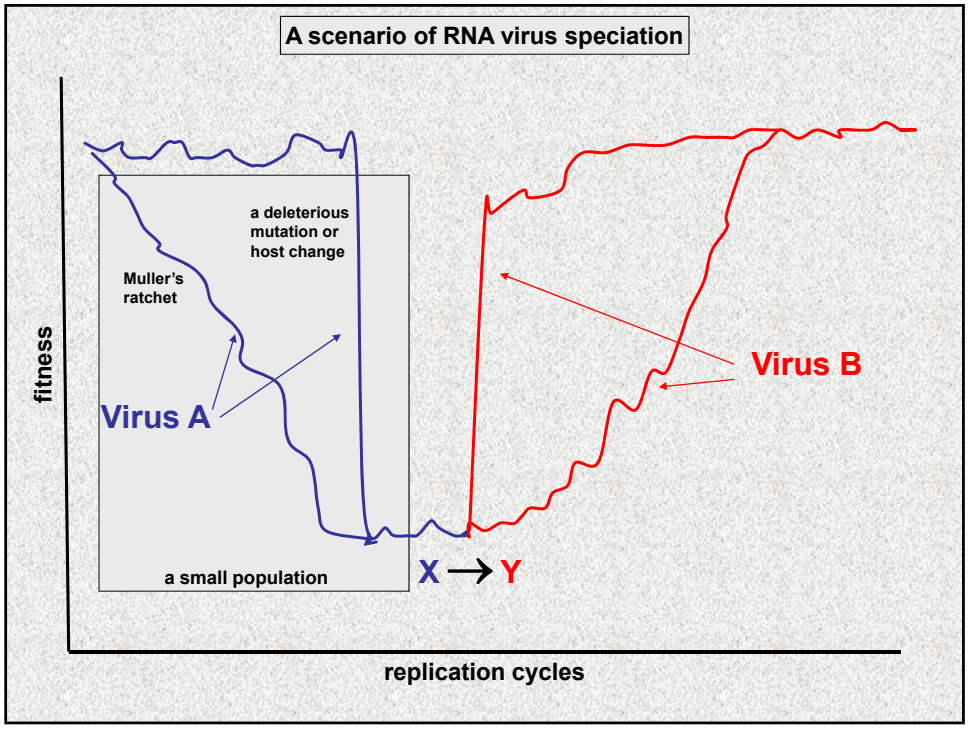
Having no selective advantage, they do not tend to accumulate.

However, when their parents are severely disabled, such recombinants may give rise to new lineages, including novel species.

Thus, the evolution operates not only by selection of the most fittest, but also may be favored by a less-fit step.

The existence of a less-fit step may be a general event in viral speciation





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HOWEVER,

Let's hope
that the knowledge
accumulated during the century of polio research
will not only force us to be skeptical,
but also will show the way to overcome
the inherent formidable properties
of the virus

Some promising approaches are already in sight

The successful outcome requires however
intensification of poliovirus research,
not its impediment
which has actually resulted from
the miscalculated containment policy of the WHO

The lessons derived from the studies on stability, variability, and evolution of the poliovirus genome make the ground for our knowledge of these aspects of molecular biology of other RNA-viruses, though some of the relevant regularities are not necessarily uniform

Thank you!