IBMP | Exploring the plant world

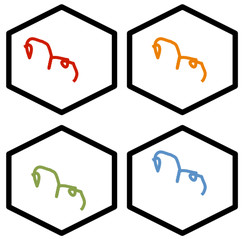
Multipartite phytovirus long distance movement:

Keep connected or die

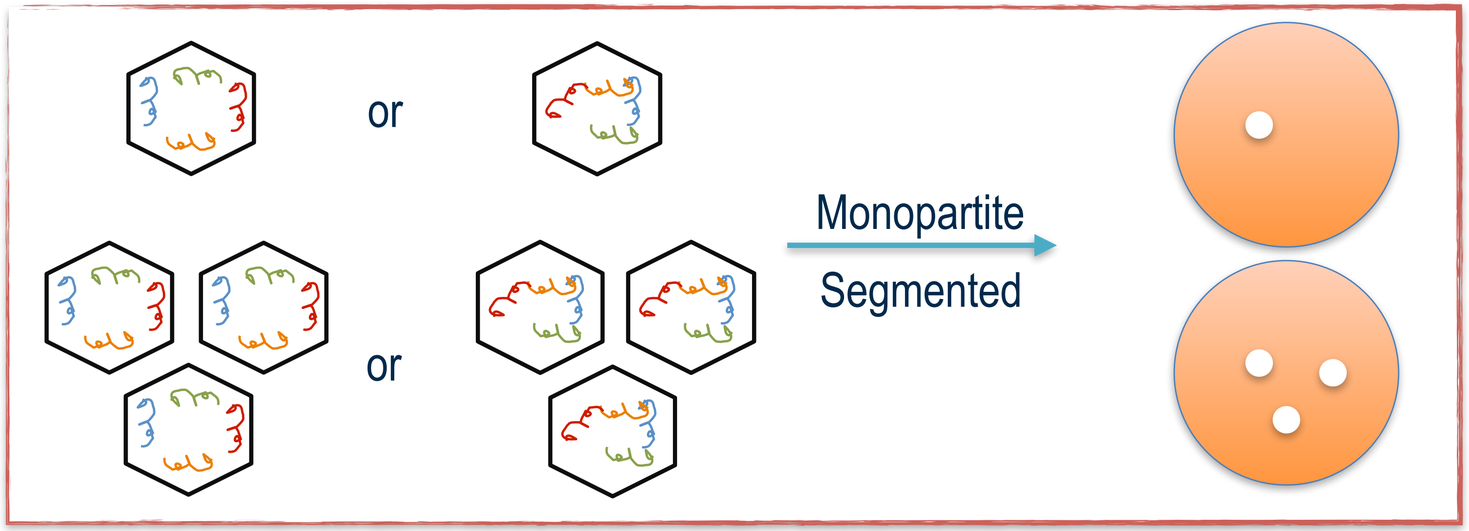
Pr. David Gilmer
Institut de biologie moléculaire des plantes
Salzburg, 7 July 2018

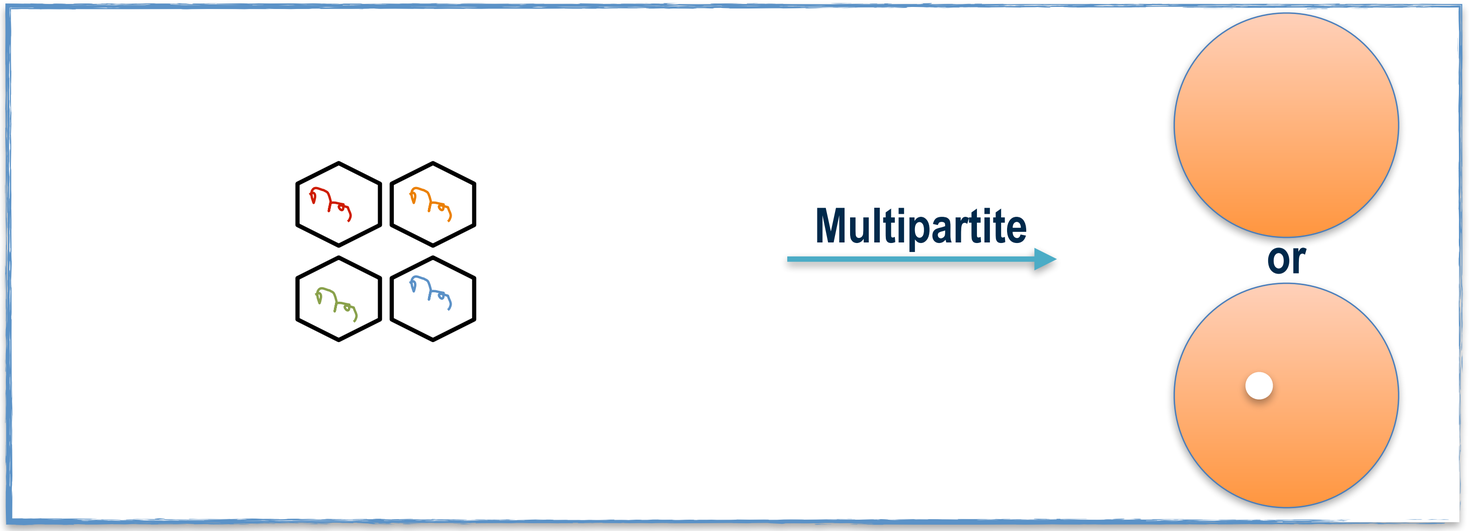
affiliated with
University of Strasbourg

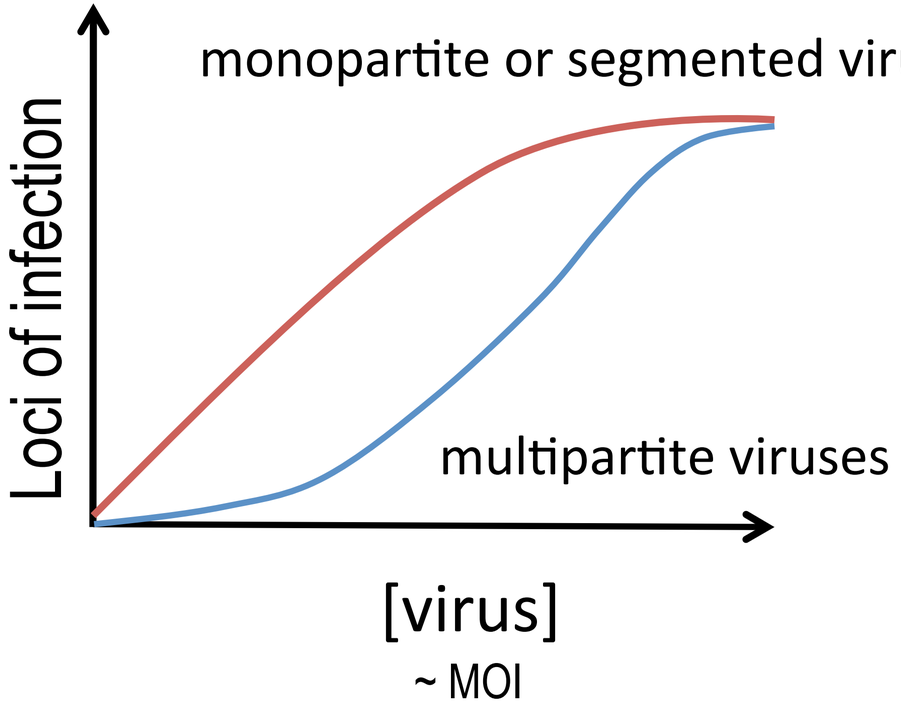
Overview of Terminology Aspects

Genome (DNA or RNA)	Virion	Designation
Monopartite	or e.g. Adenovirus; e.g. Tobacco mosaic virus	Monopartite
Multipartite: Segmented	e.g. Influenza A virus (8 segments)	Segmented
Multipartite: Segmented	One/many kinds (=> increased virion stability; Ojosnegros et al. 2011)  or e.g. *Nanoviridae* (6 to 8); e.g. Beet necrotic yellow vein virus (4 to 5)	**Multipartite**

One full genome / One infection







Benefits vs Costs

Viral genome = fixed number of each gene

Replication cycle : « cell autonomous » and spread to neighbour cells

Adaptation to environment through sequence mutation(s)

10 kb

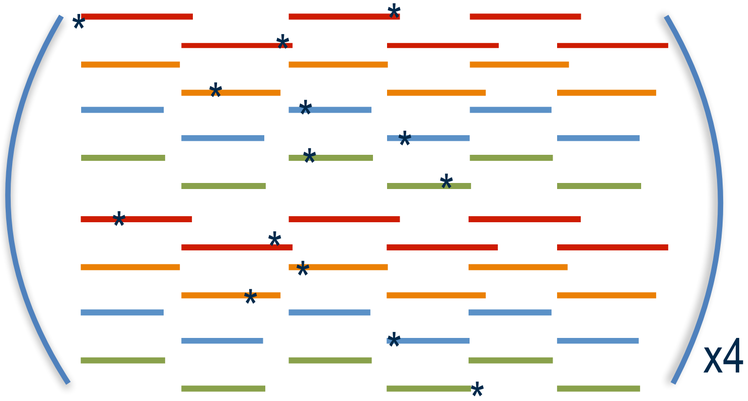
Replication time

* mutation rate ~10^{-4}

Genetic exchange requires **recombination**

∑10 kb

Replication time÷4

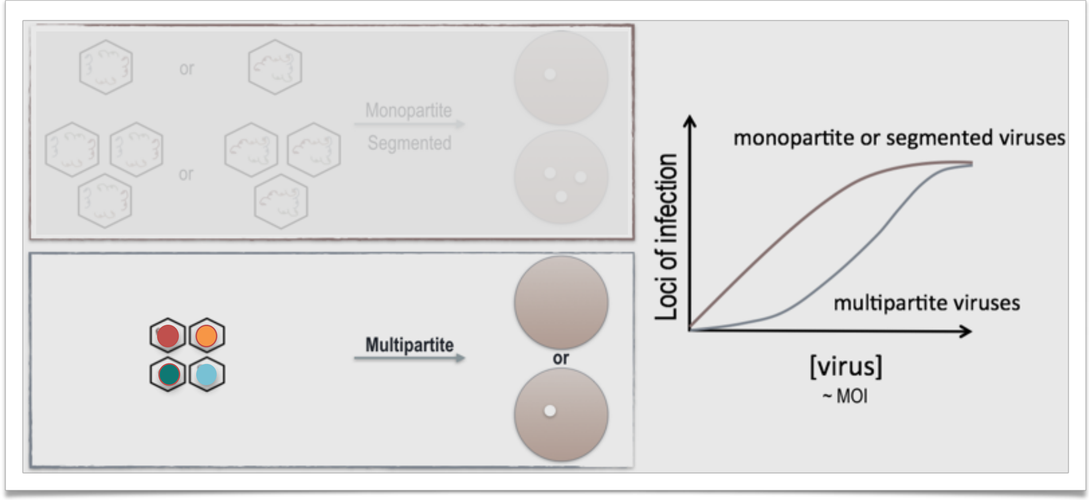


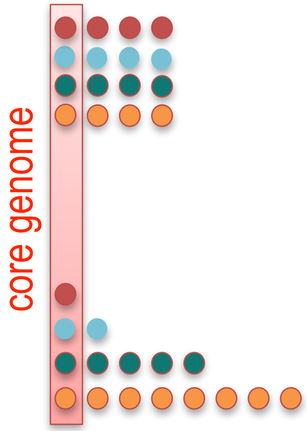
x4

Genetic exchange favoured by segment **reassortment**

Costs for multipartite viruses : what is the minimum ?

Probability of **infection decreases** with the number of genomic segments and further decreases with unequal frequencies





equal frequencies
max ~ 4

unequal frequencies
max ~ 1

~~**Virion stability**~~
~~**Replication time**~~

Advantages are not dependant on frequencies

~~* mutation escape~~
~~Favoured **exchange**~~

The different genomic segments should « accumulate » to equal frequencies for a minimal cost

Gene-accordions // (gene) segment frequency variation: convergent regulatory/adaptative ?

4 - 8 July 2018
Salzburg - Austria
EVOLUTION
Genetic Novelty/Genomic Variations by RNA Networks and Viruses

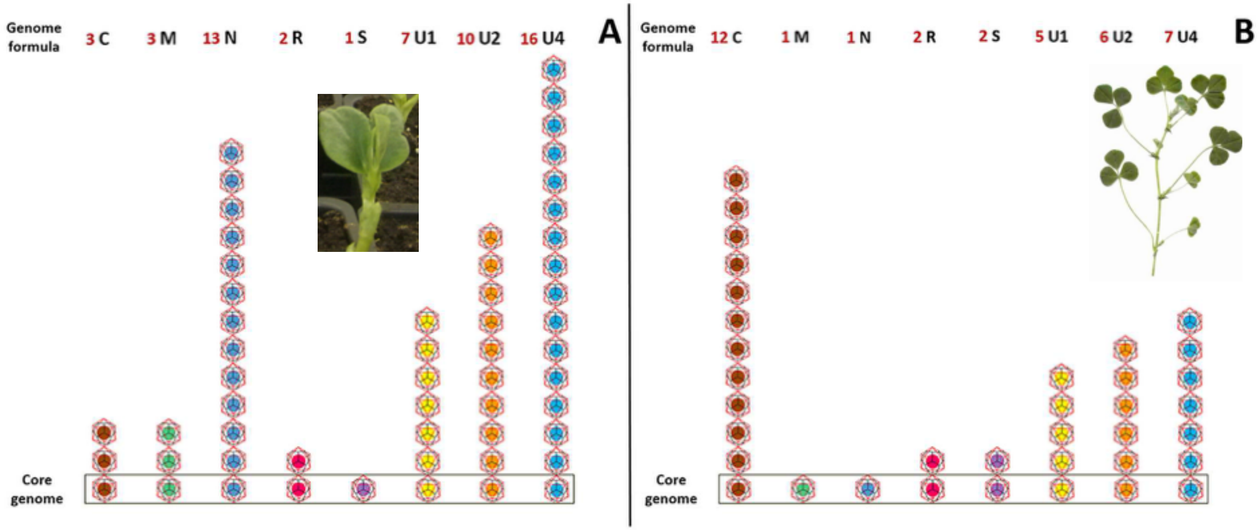
The Strange Lifestyle of Multipartite Viruses

Anne Sicard[1], Yannis Michalakis[2], Serafín Gutiérrez[3], Stéphane Blanc[1]*

PLOS | PATHOGENS

Nanovirus
Faba bean necrotic stunt virus
(8 ssDNA)

Segments accumulation is not equal and depend on host



2016 - PLoS Pathos 12(11): e1005819

Poxviruses Deploy Genomic Accordions to Adapt Rapidly against Host Antiviral Defenses

Nels C. Elde,[1,4,*] Stephanie J. Child,[2] Michael T. Eickbush,[1] Jacob O. Kitzman,[5] Kelsey Adam P. Geballe,[2,3,6,7] and Harmit S. Malik[1,8,*]

In contrast to RNA viruses, double-stranded DNA viruses have low mutation rates yet must still adapt rapidly in response to changing host defenses. To determine mechanisms of adaptation, we subjected the model poxvirus vaccinia to serial propagation in human cells, where its antihost factor K3L is maladapted against the antiviral protein kinase R (PKR). Viruses rapidly acquired higher fitness via recurrent K3L gene amplifications, incurring up to 7%–10% increases in genome size. These transient gene expansions were necessary and sufficient to counteract human PKR and facilitated the gain of an adaptive amino acid substitution in K3L that also defeats PKR. Subsequent reductions in gene amplifications offset the costs associated with larger genome size while retaining adaptive substitutions. Our discovery of viral "gene-accordions" explains how poxviruses can rapidly adapt to defeat different host defenses despite low mutation rates and reveals how classical Red Queen conflicts can progress through unrecognized intermediates.

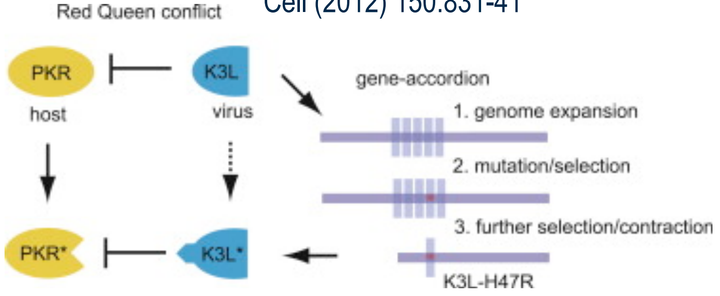
Cell (2012) 150:831-41

[Biochem. J. (2000) 346, 17–24 (Printed in Great Britain)]

Quantitative analysis of gene amplification in insecticide-resistant *Culex* mosquitoes

Michael G. PATON, S. H. P. Parakrama KARUNARATNE, Elsa GIAKOUMAKI, Neil ROBERTS and Janet HEMINGWAY[1]
School of Biosciences, University of Wales Cardiff, P.O. Box 915, Cardiff CF1 3TL, U.K.

The amplification of carboxylesterase structural genes followed by their overexpression is the most common mechanism of resistance to organophosphorus insecticides in *Culex* mosquitoes. Most resistant *Culex quinquefasciatus* mosquitoes have co-amplified *est*α*2*[1] and *est*β*2*[1] genes. Recently, Southern, DNA dot-



Beet necrotic yellow vein virus (BNYVV)

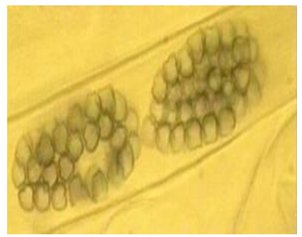
Most segmented genome among (+) strand RNA viruses
Multipartite virus
Each capped and poly-A viral genomic RNA **individually packaged into distinct rod-shaped particles**

The VIRUS

Benyviridae *Benyvirus* *Beet necrotic yellow vein virus*

Rhizomania

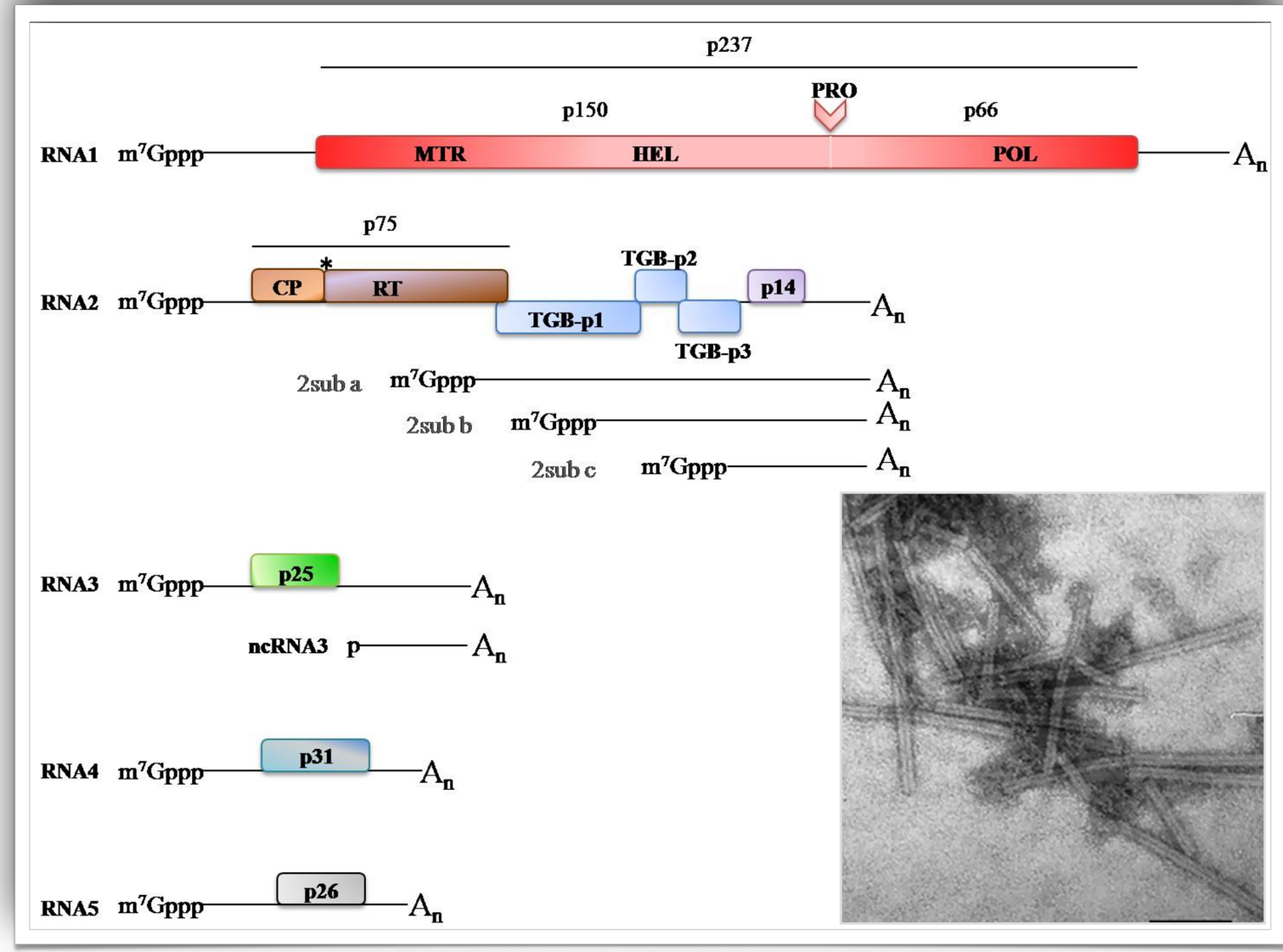
The VECTOR



Polymyxa beta
(Protozoa)

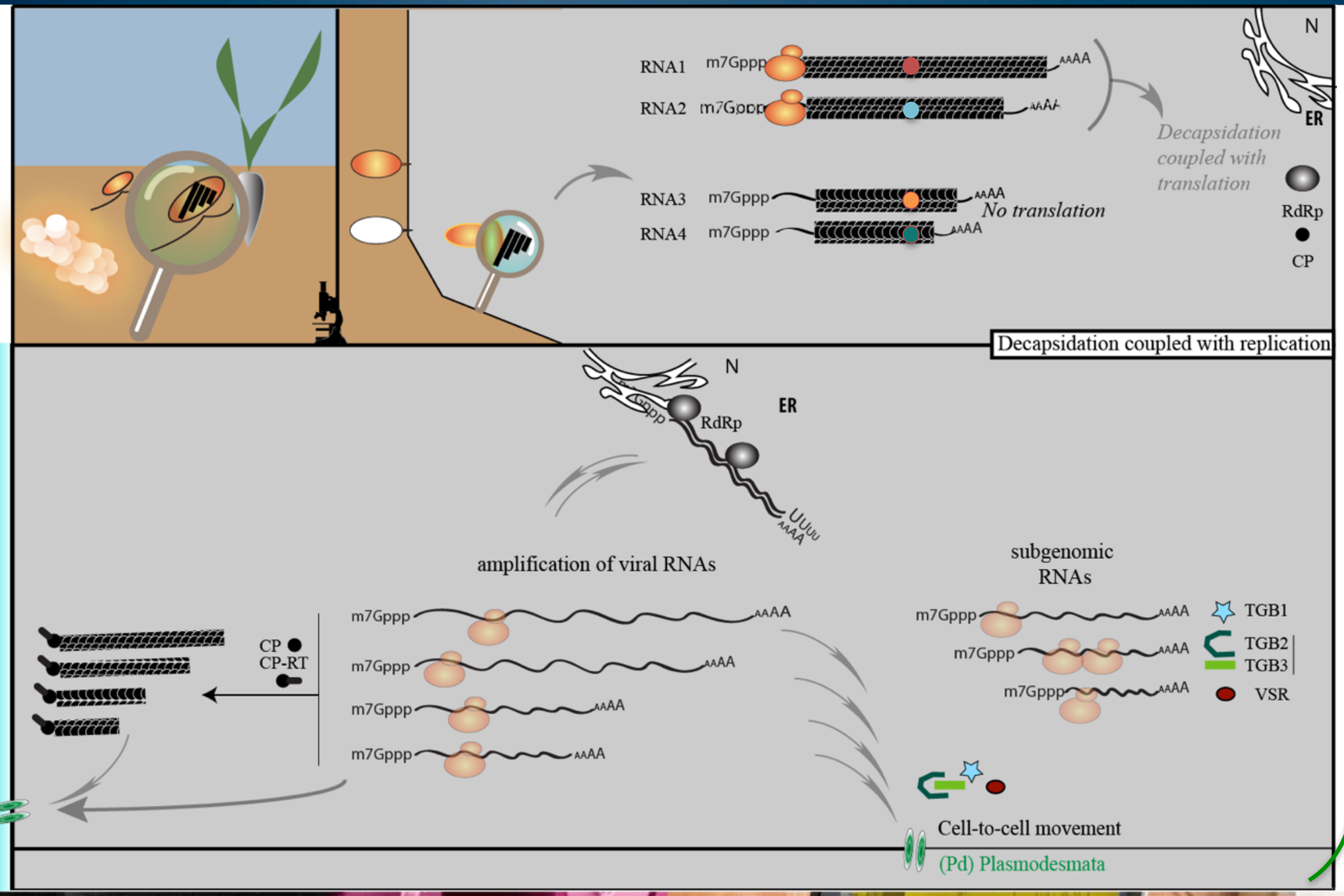
The HOST

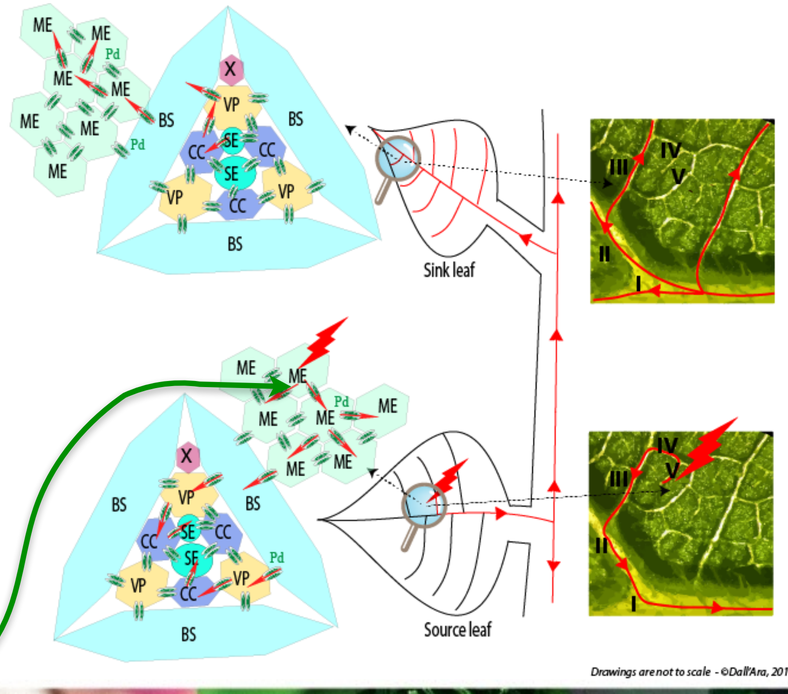
Beta vulgaris sp.



A ‘textbook’ view for a plant multipartite RNA lifecycle (e.g. BNYVV)

4 - 8 July 2018
Salzburg - Austria
EVOLUTION
Genetic Novelty/Genomic Variations by RNA Networks and Viruses

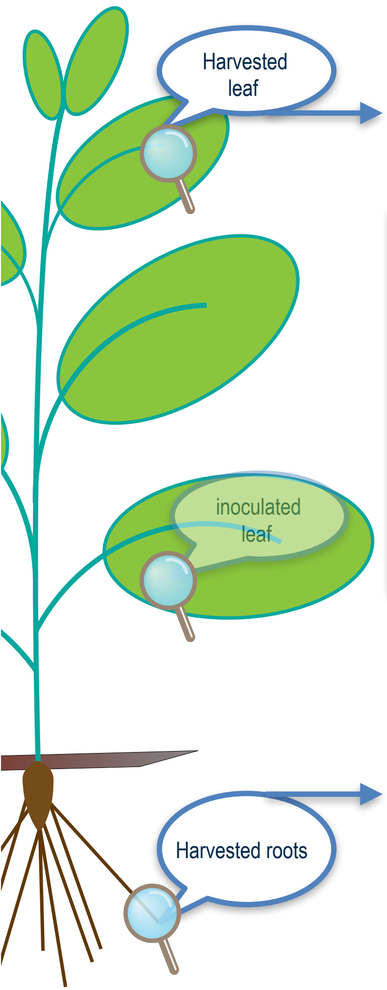


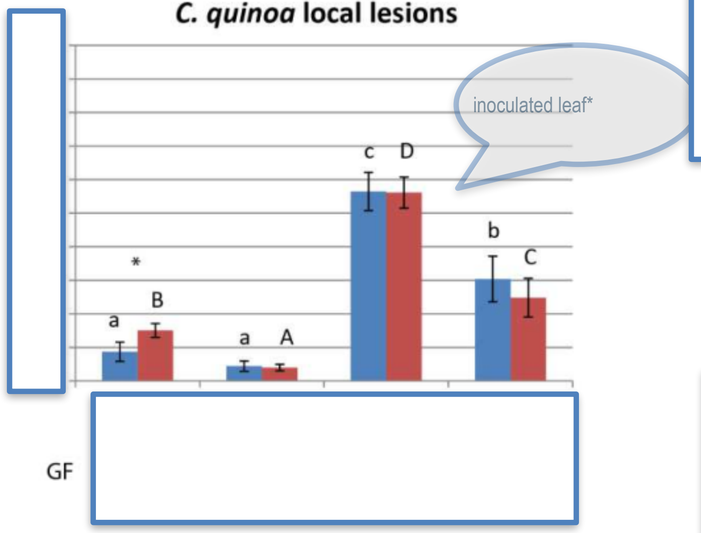


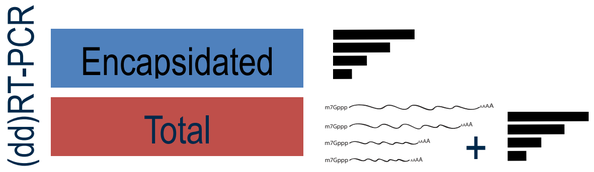
a *Déjà vu* impression

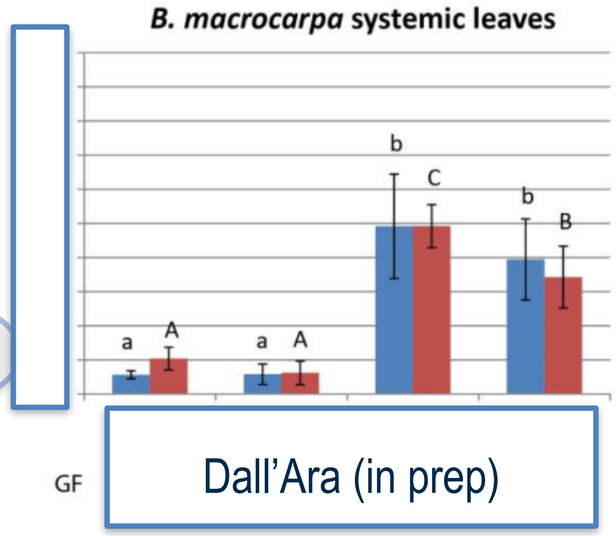
BNYVV segment **relative** frequencies in infected plant tissues

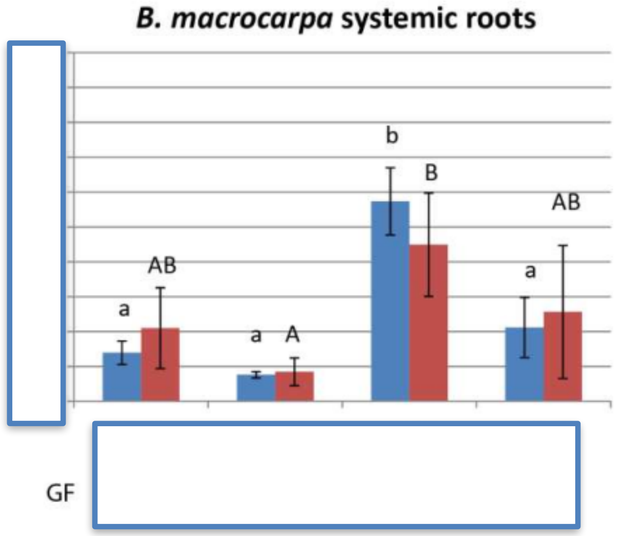
4 - 8 July 2018
Salzburg - Austria
EVOLUTION
Genetic Novelty/Genomic Variations by RNA Networks and Viruses





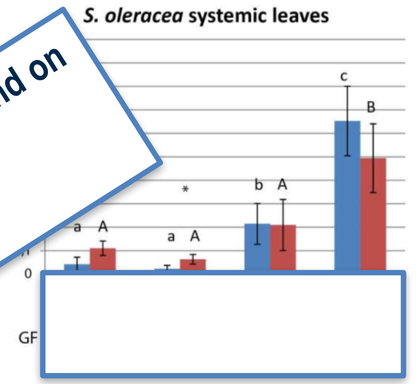


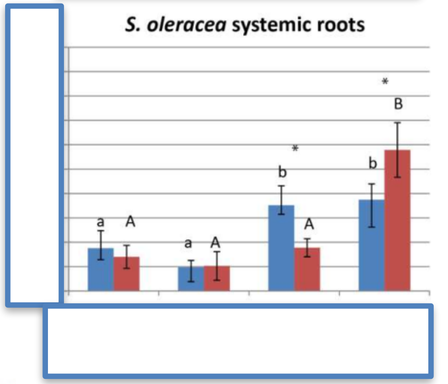




Segment frequencies differ mean ~ $X^{RNA1}/Y^{RNA2}/Z^{RNA3}/A^{RNA4}$ = genome formula

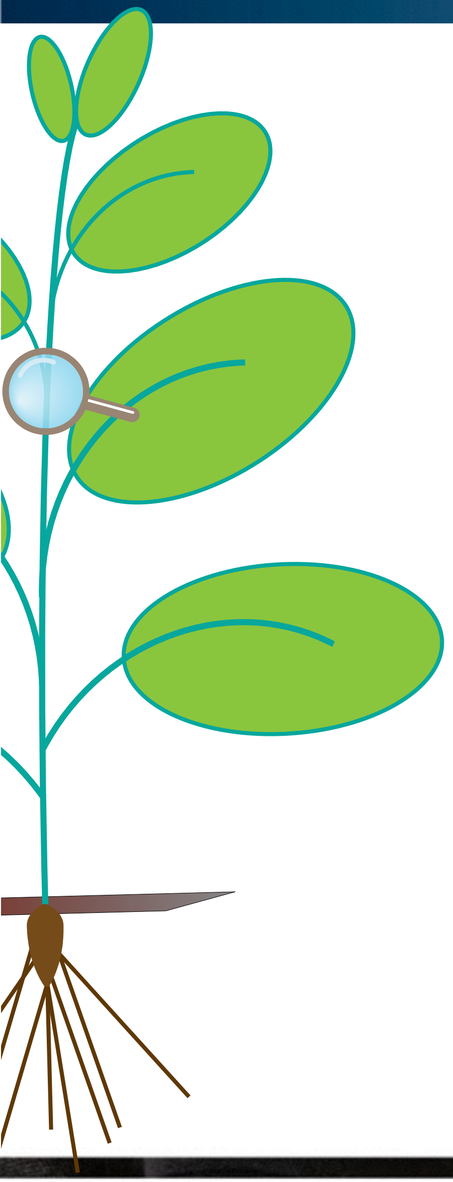
Segment frequencies depend on
Host
Tissue

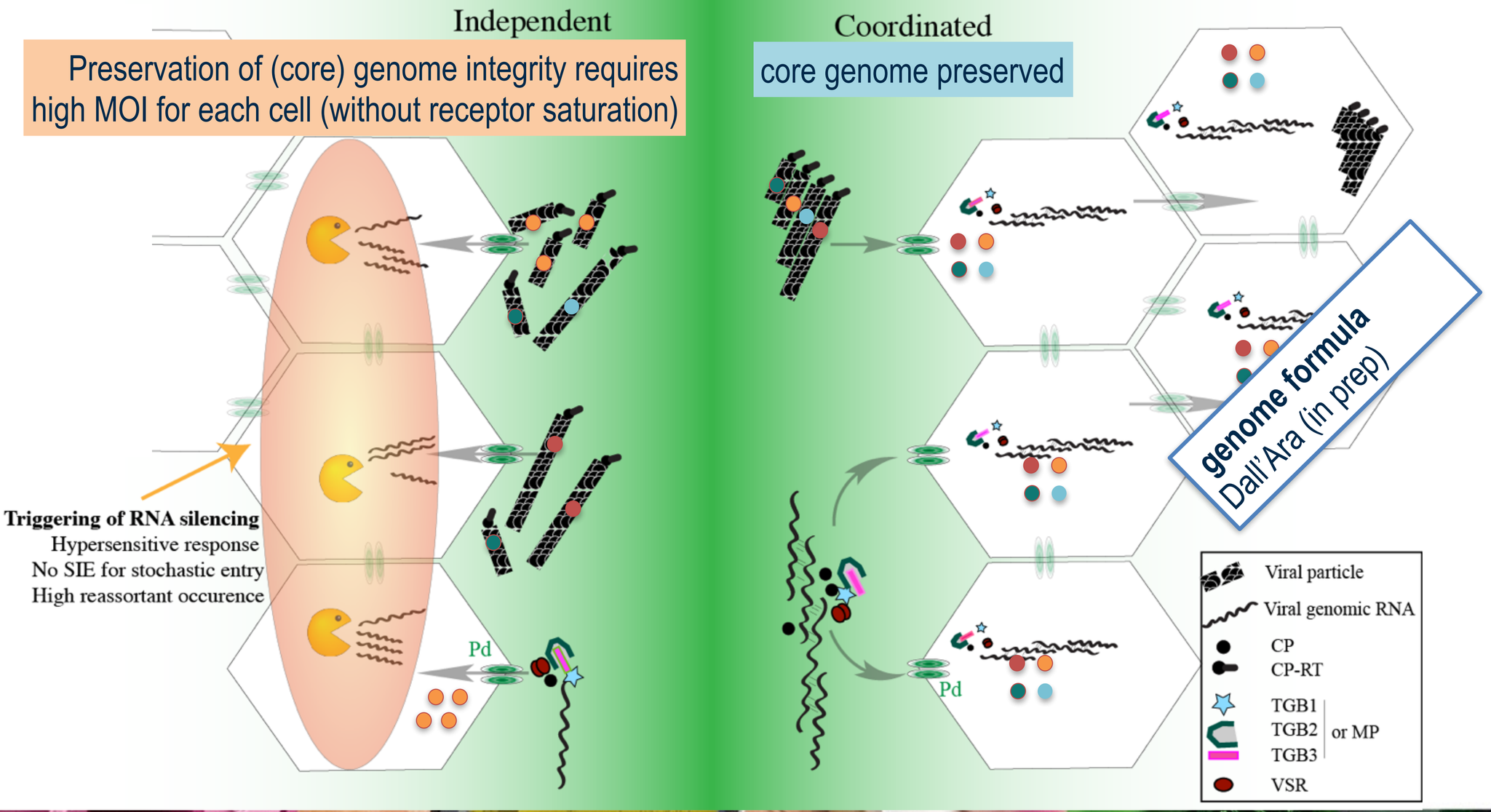




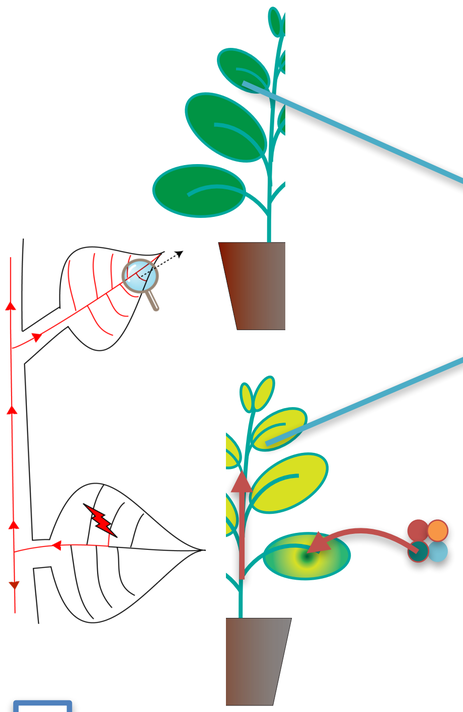
What is moving in plant vascular tissues, how and what consequences ?

4 - 8 July 2018
Salzburg - Austria
EVOLUTION
Genetic Novelty/Genomic Variations by RNA Networks and Viruses

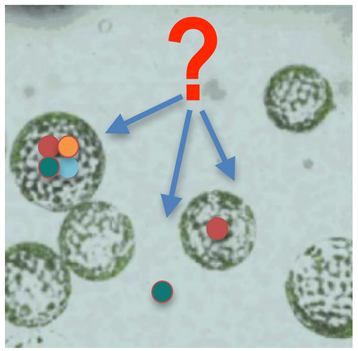




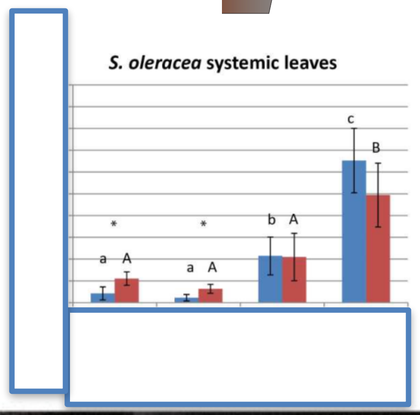
Segments are mainly present altogether in single cells

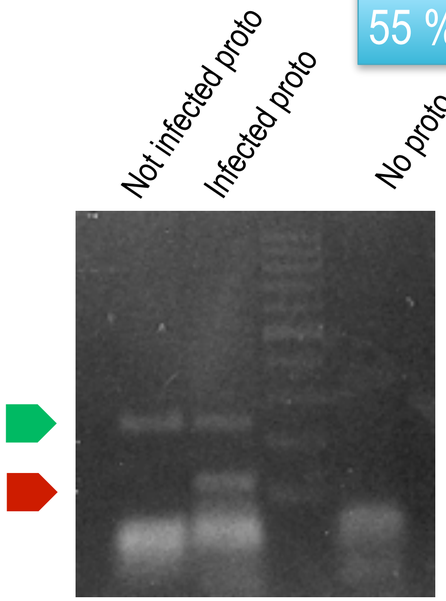


Mix of Infected / Healthy isolated cells



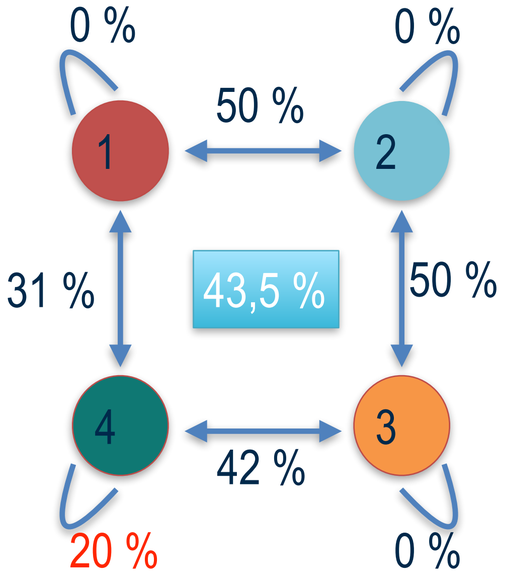
dilution/fixation
Sampling
(± 1 cell / multiplex
RT-PCR BNYVV - Rubisco)





55 % with no viral signal

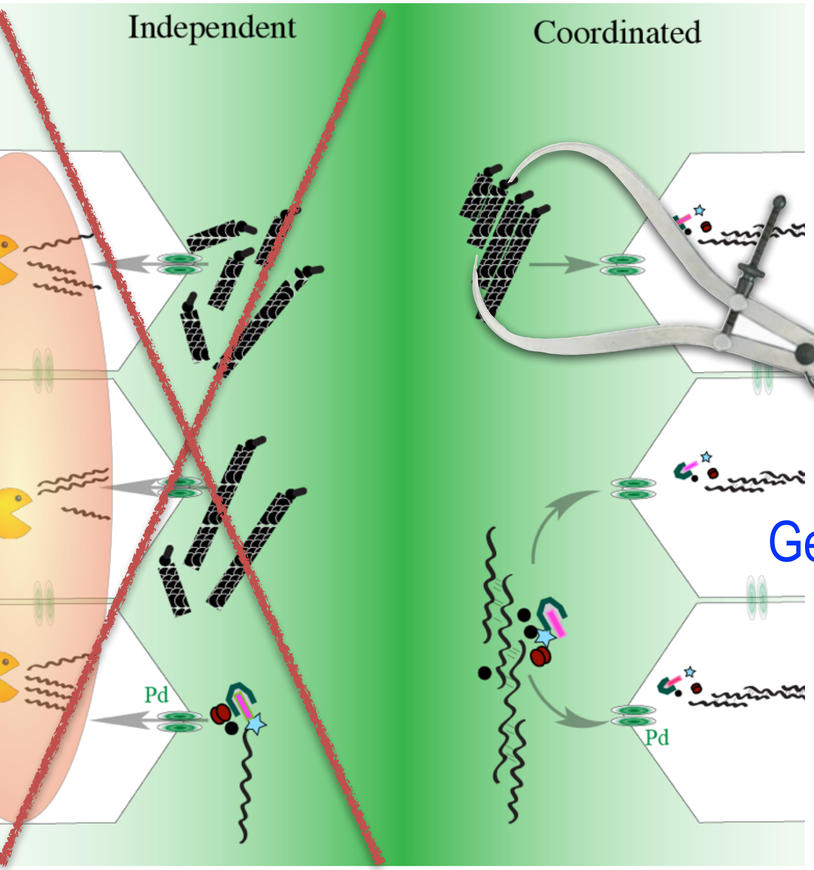
∑rubisco⁺ & no signal /∑ rubisco⁺



A coordinated movement of a collective infective unit insure genome integrity

Dall'Ara (in prep)

BNYVV long distance movement:
A Coordination that requires selection



Virion selection will require molecular Callipers to compile at least one of each size

BNYVV CP is required for long distance movement
BUT encapsidation is not mandatory

Genomic RNA selection will require specific RNA interactions to create an RNA network

Long stretches of perfect base pairing can trigger RNA silencing

Finding the needle in a haystack

How to find needles in a haystack without a magnet ?

C. quinoa

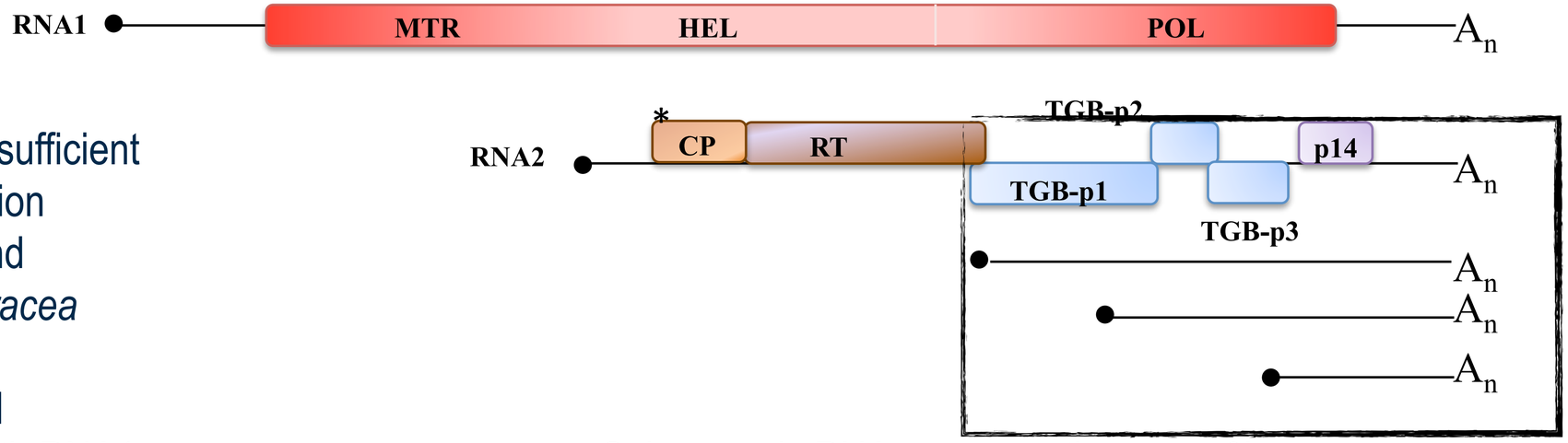
Local infection

RNA1 and RNA2 are sufficient for a productive infection locally in *C. quinoa* and systemically in *S. oleracea*

We suspect a minimal interaction network with RNA1 and RNA2

S. oleracea

Systemic infection



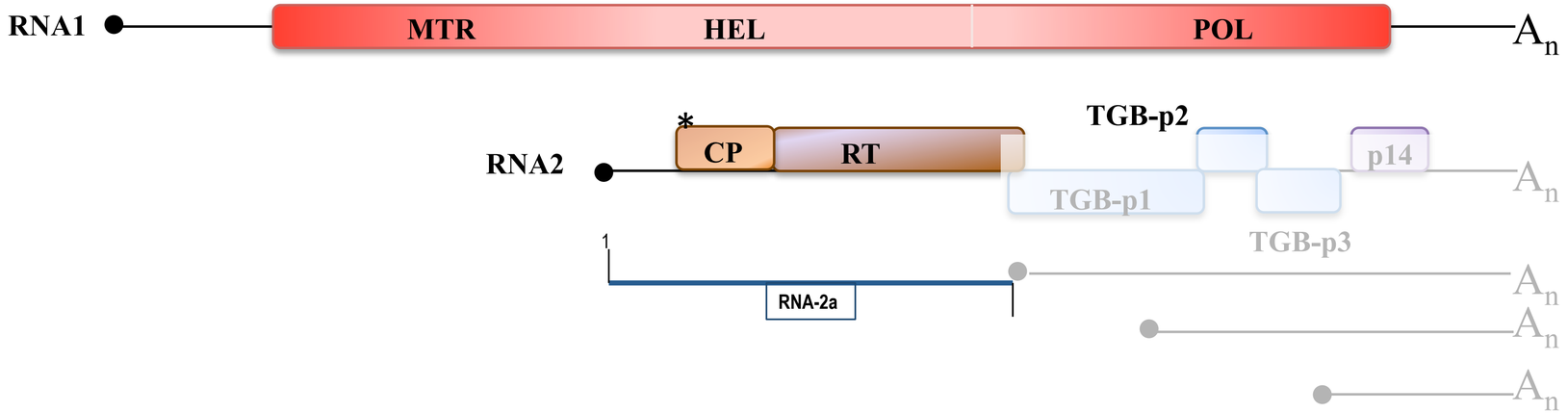
Subgenomic RNAs possess the same 3' sequence : possible interference with the network

The interaction(s) domain(s) should be present within the 5' region of RNA2

How to find needles in a haystack without a magnet ?

C. quinoa

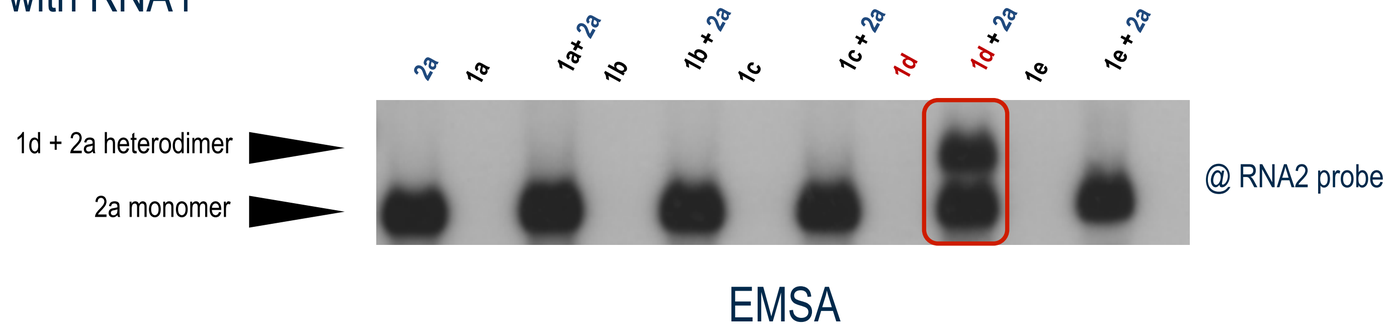
Local infection



We suspect a minimal interaction network with RNA1 and RNA2

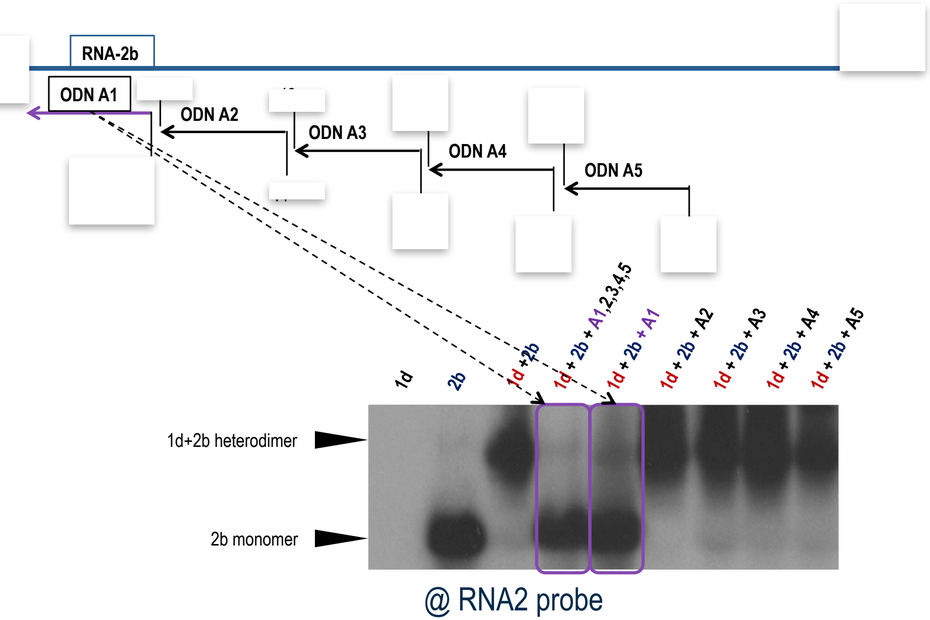
S. oleracea

Systemic infection

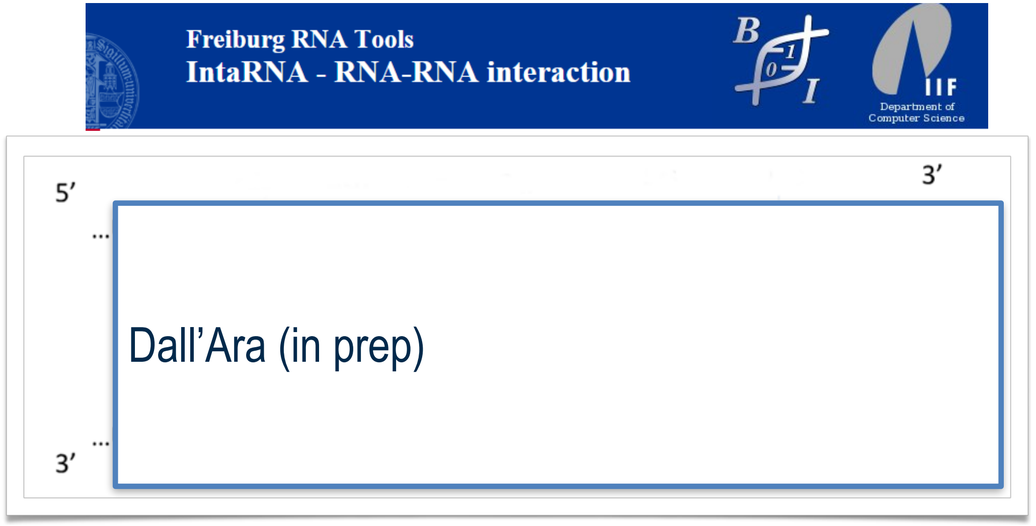


EMSA

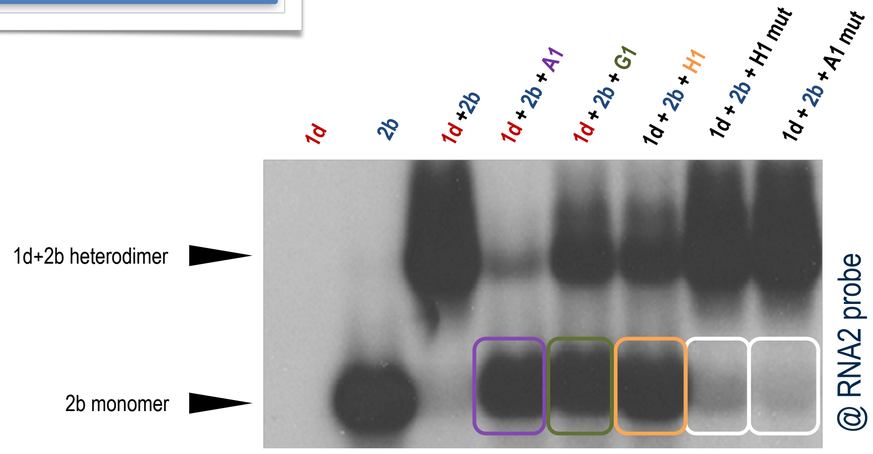
How to find needles in a haystack without a magnet ?



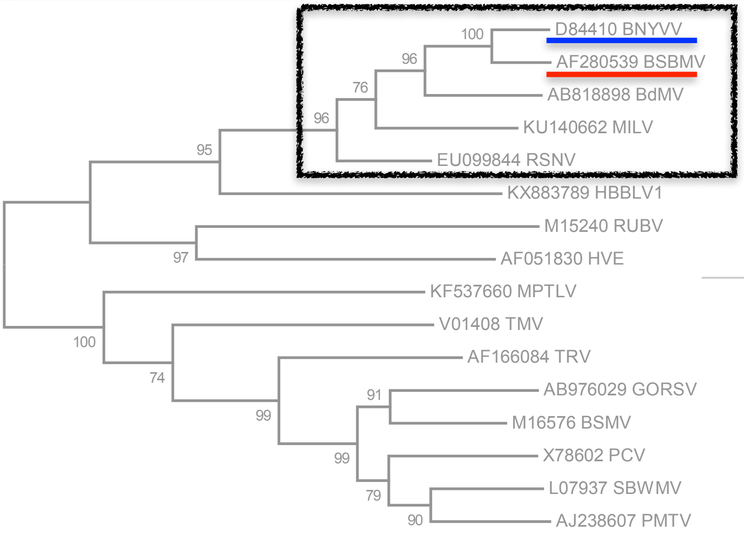
@ RNA2 probe



Dall'Ara (in prep)

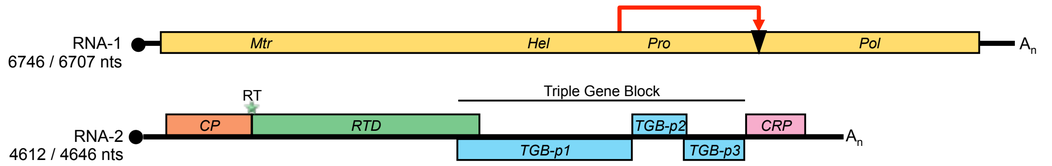


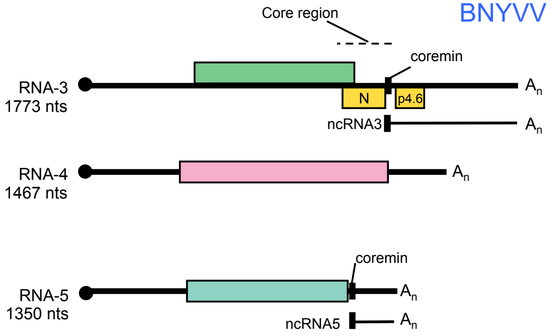
Use of phylogeny

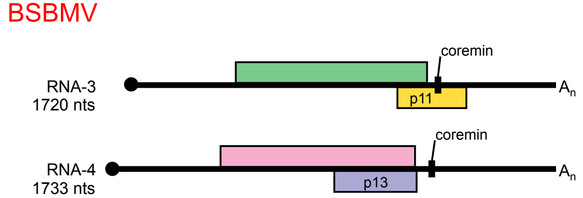


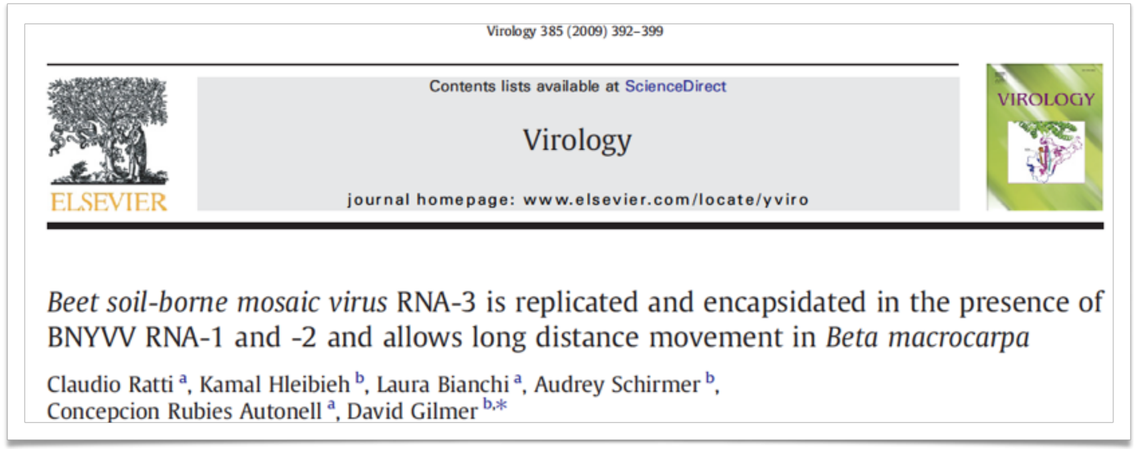
BNYVV/BSBMV distribution in USA

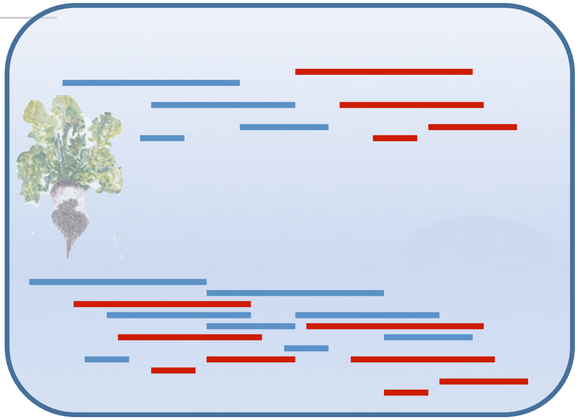
BNYVV / BSBMV

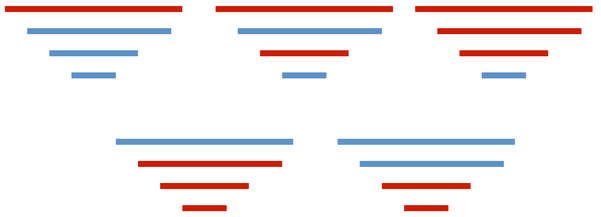












No such genetic exchanges found in nature so far

Ressortants are viable

RNA-1 BNYVV
RNA-2 BNYVV

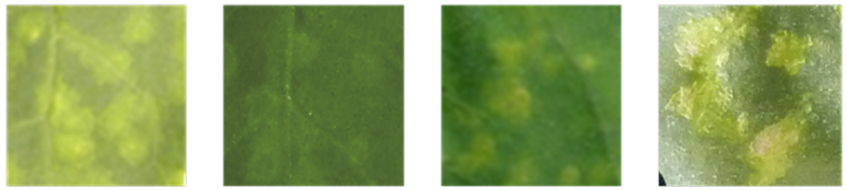
RNA-1 BSBMV
RNA-2 BSBMV

BS helper strain

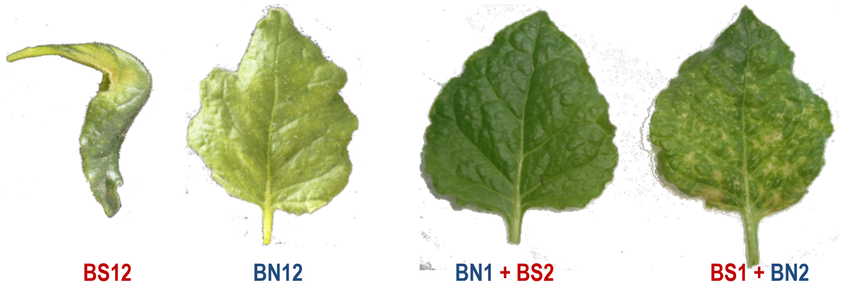
RNA-1 BNYVV
RNA-2 BSBMV

RNA-1 BSBMV
RNA-2 BNYVV

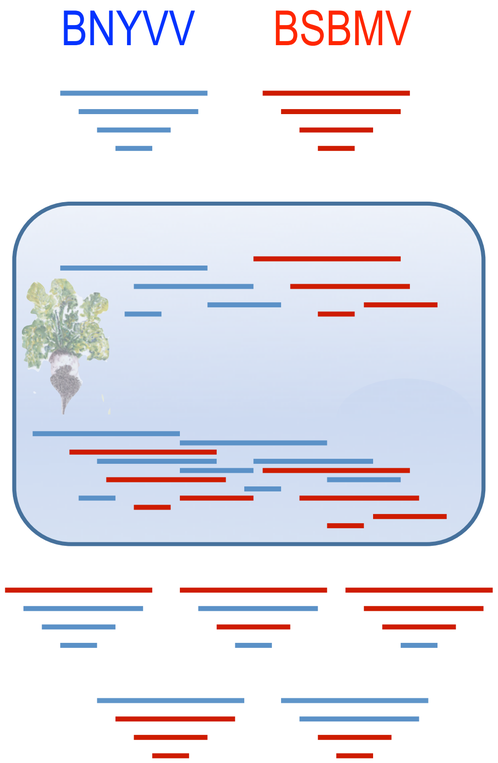
BS1 +BN2

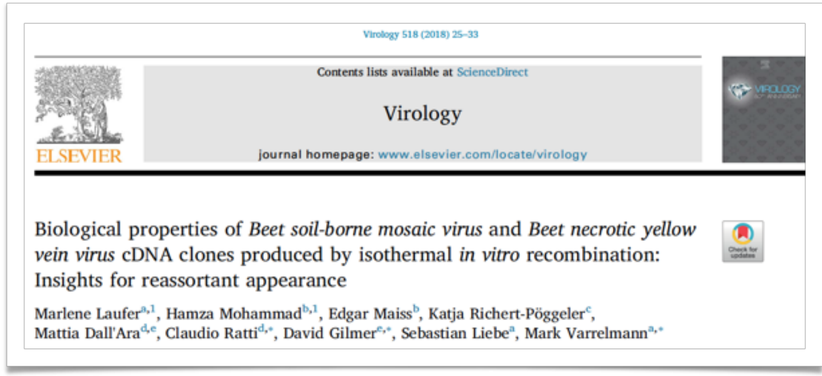


BS12 BN12 BN1 + BS2 BS1 + BN2



BS12 BN12 BN1 + BS2 BS1 + BN2





Virology 518 (2018) 25–33

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

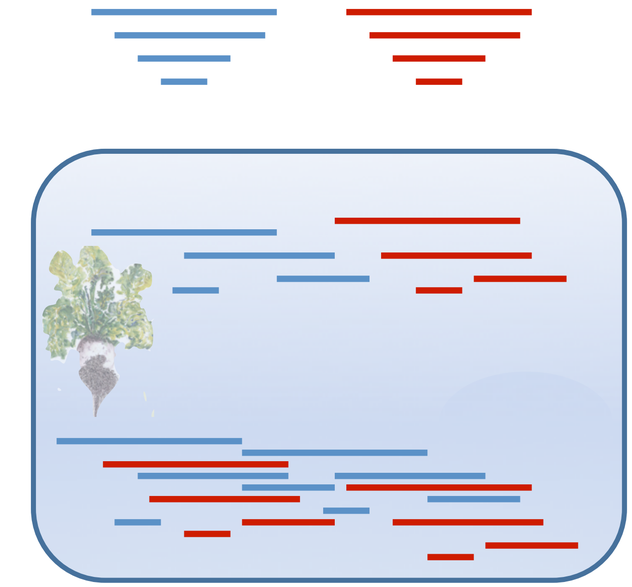
Biological properties of *Beet soil-borne mosaic virus* and *Beet necrotic yellow vein virus* cDNA clones produced by isothermal *in vitro* recombination: Insights for reassortant appearance

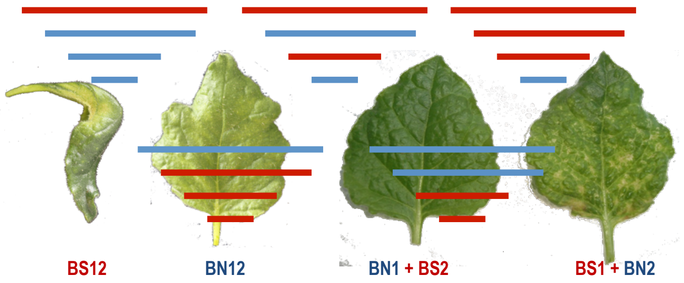
Marlene Laufer[a,1], Hamza Mohammad[b,1], Edgar Maiss[b], Katja Richert-Pöggeler[c], Mattia Dall'Ara[d,e], Claudio Ratti[d,*], David Gilmer[e,*], Sebastian Liebe[a], Mark Varrelmann[a,*]

Reassortants are viable and functional

Towards a functional validation

BNYVV BSBMV

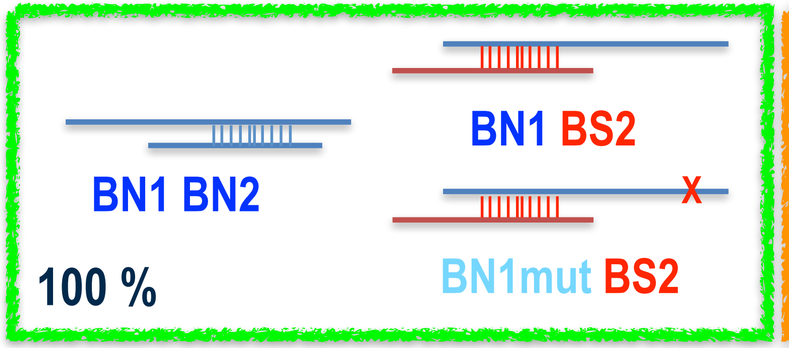


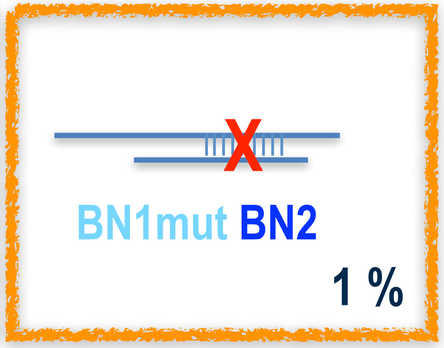


5′ Dall'Ara (in prep) 3′

BN1 ~~BN2mut~~

RIP (in protoplasts)





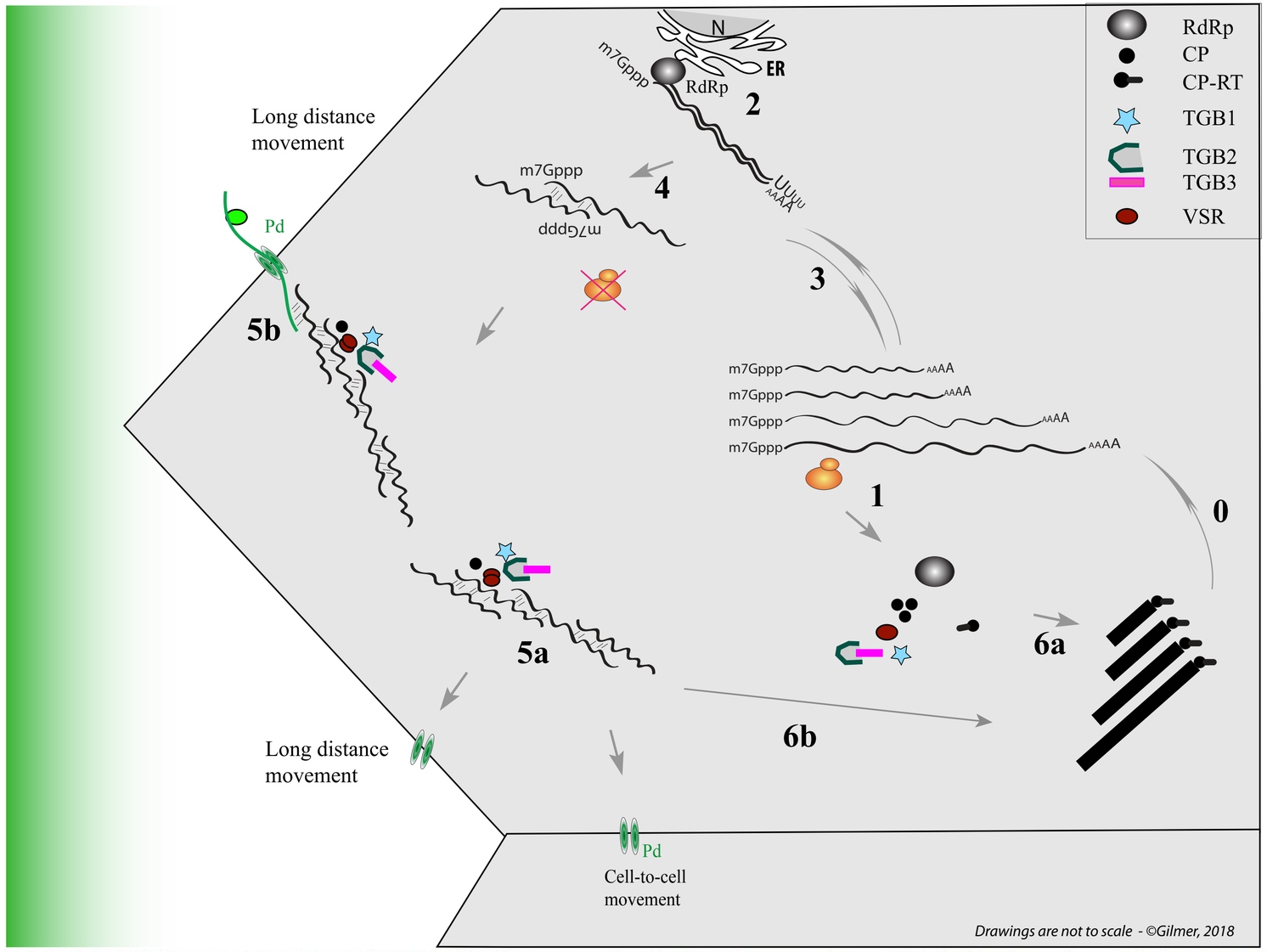
Kissing interactions affect BNYVV biology

1BN	2BN	2BS	1BS	2BN	2BS
√	√	X	√	X	√

Genomic RNAs are selectively chosen for long distance movement

‘Revisited’ view for a plant multipartite RNA lifecycle (e.g. BNYVV)

4 - 8 July 2018
Salzburg - Austria
EVOLUTION
Genetic Novelty/Genomic Variations by RNA Networks and Viruses



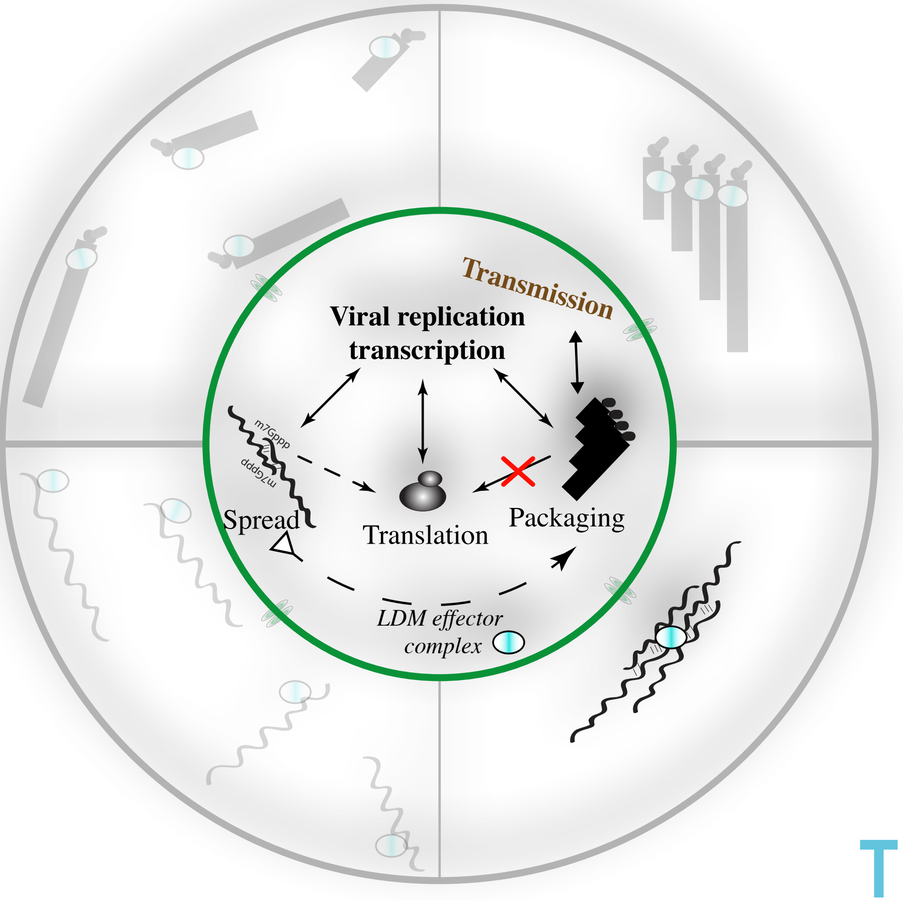
Steps

0: Unpacking
1: Translation
2-3: Replication/Transcription
5: Cell-to-cell movement
6: Packaging

revisited steps

4: genomic RNA-RNA interactions regulating viral expression (on/off) and RNP network formation for cell-to-cell and long distance movement (**5**)

5a RNP complex is autonomous
5b RNP complex could be driven by moving cellular mRNAs (e.g. Knotted-1)



Acknowledgements:

Dr. Mattia **Dall'Ara** & Dr. Claudio **Ratti** (Bologna, It)

Dr. Alyssa Flobinus, Dr. Salah Bouzoubaa, Dr. Fabrice Michel,

Dr. Isabelle Lebars, Dr. Eric Ennifar & Dr. Valérie Vivet (CNRS/Strasbourg)

Elodie Klein (Engineer, SES), Danièle Scheidecker (Engineer), Lucie Bellott (PhD), Réjane Helfer (Master)

Dr. Stéphane **Blanc** (Montpellier), brainstorming

Thank you for your attention