

‘Variation in the p25 region of resistance breaking *Beet necrotic yellow vein virus* from infected plants in Minnesota’

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- Tolerance to BNYVV conferred by the *Rz1* loci (inherited as a single dominant gene)
- *Rz1* has been widely deployed in resistant cultivars from the mid 1980s onwards
- In 2002-2003 BNYVV tolerant cultivars showed severe rhizomania symptoms in Minnesota

# Symptoms of BNYPVV



Lateral root proliferation and  
tap root constriction



Healthy

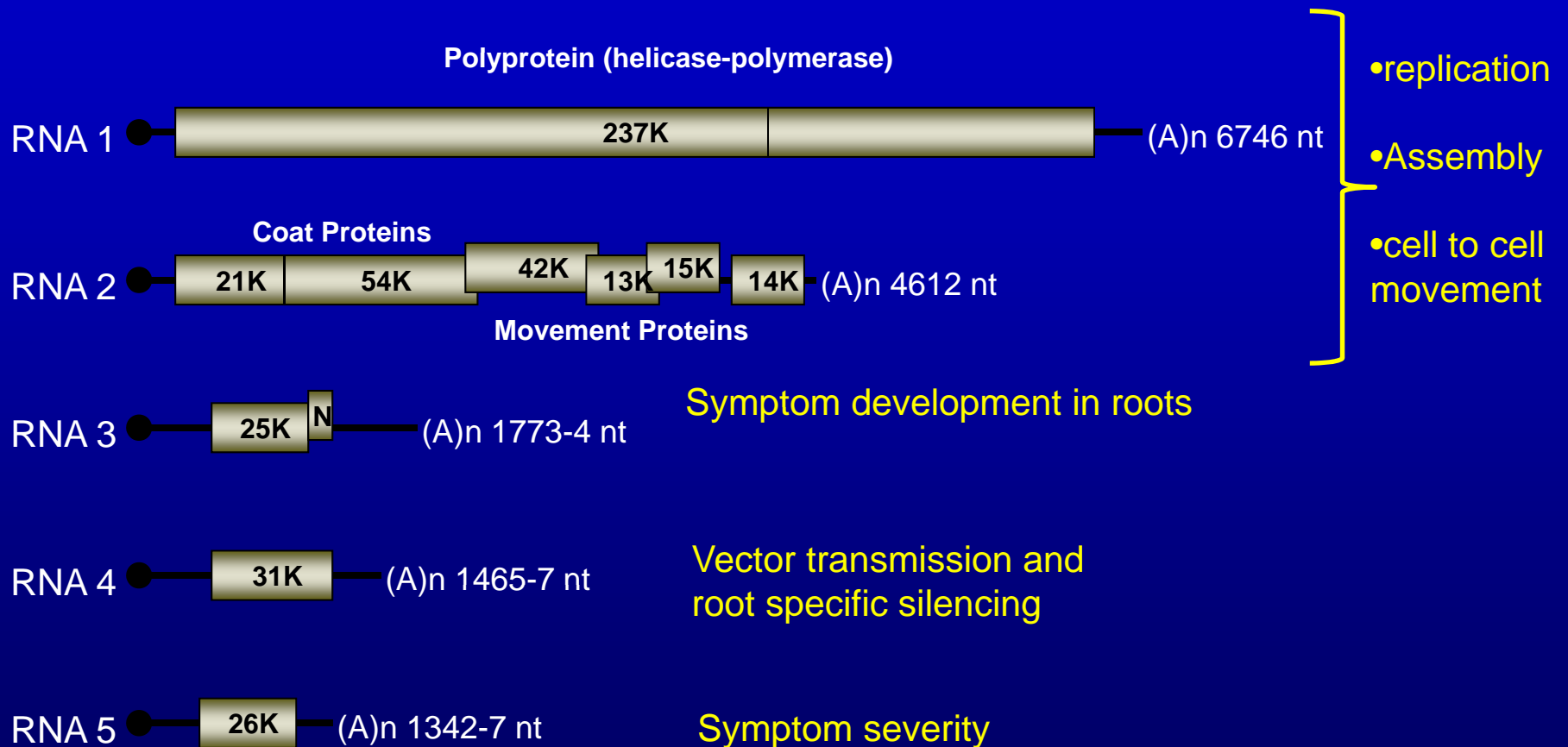
Diseased

# BNYVV Symptoms - field



- How and why is BNYVV able to overcome this resistance?
- Is there an underlying reason for the epidemiological differences seen in field distribution?

# Beet necrotic yellow vein virus (BNYVV)

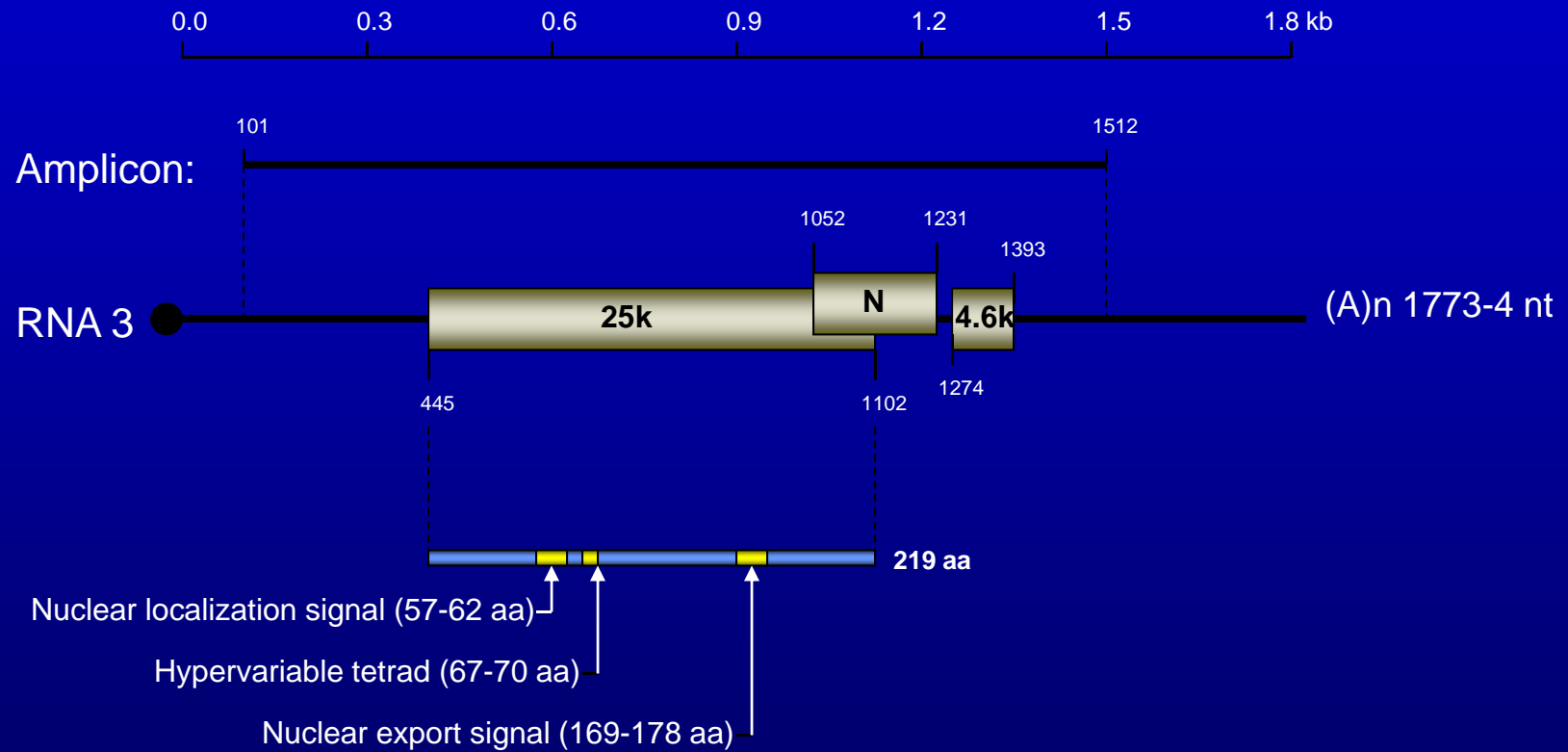




# Amino Acid Motifs In the p25 of BNYVV

- Reverse genetics approach by Koenig *et al.* (1991) showed this region is important for pathogenicity of BNYVV
- In addition p67 and p68 in the p25 protein of BNYVV are correlated with resistance breaking (Tamada *et al.* 2011, Koenig *et al.* 2009)

# BNYVV RNA 3





# Methodology

Sampled roots from symptomatic and asymptomatic plants

Blinkers and spots

Amplify and sequence the p25 region of BNYVV



# Methodology

- Important to sample plants rather than soil
- Important to sample symptomatic and asymptomatic plants
- Test for the presence of the *Rz* gene



# Consensus sequencing

Isolate	A. A. Motif p67	A. A. Motif p68
Minnesota WT	A	C
Minnesota RB	V	C
California WT	A	C
California RB	V	L

R. Acosta –Leal and Rush 2007

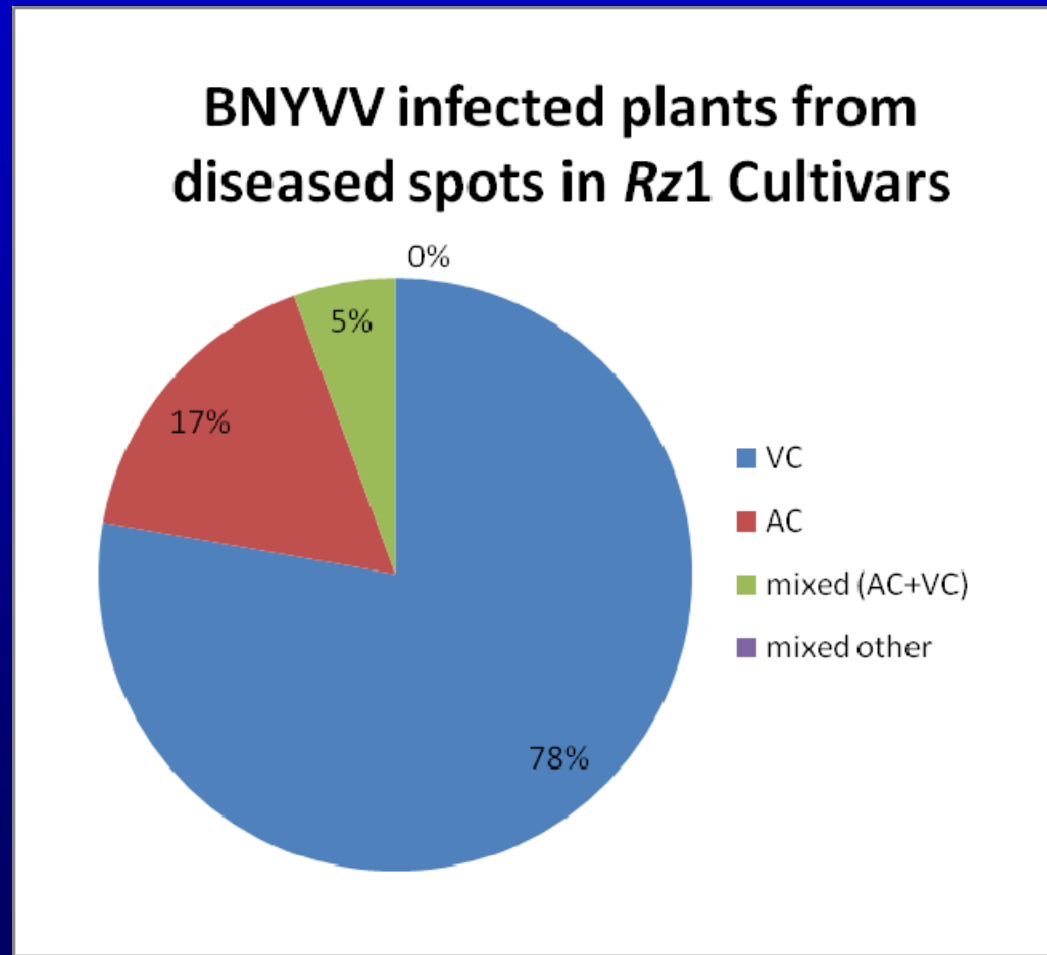
# Amino Acids at p 67 and 68 in symptomatic plants from Minnesota spots

Plant Cond.	Location	R loci	p67	p68
yellow	spot	Rz1	V	C
yellow	spot	Rz1	A	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	A	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V & A	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	A	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C
yellow	spot	Rz1	V	C

# Amino Acids at p 67 and 68 in symptomatic plants from Minnesota Blinkers

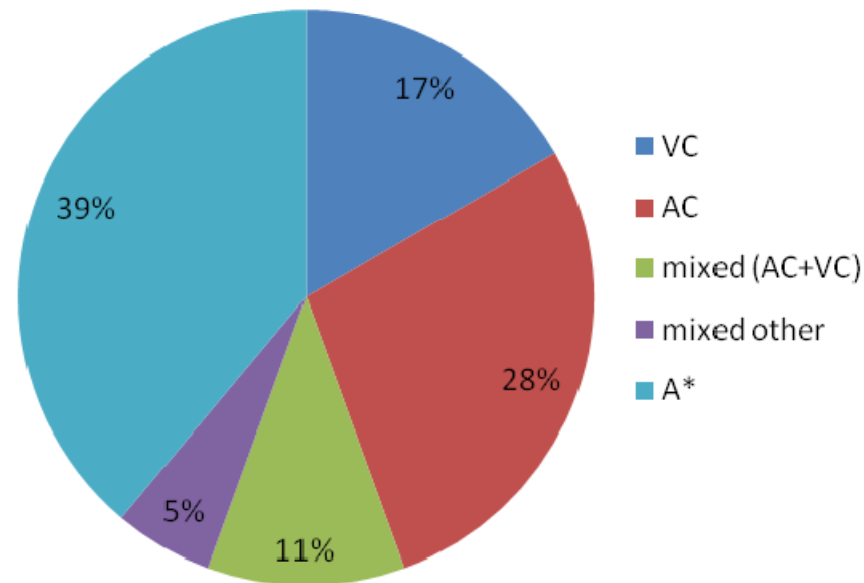
Plant Cond.	type	R loci	p67	p68
yellow	Blinker	Rz1	A	C
yellow	Blinker	Rz1	A	C & H
yellow	Blinker	Rz1	A	H
yellow	Blinker	Rz1	A	H
yellow	Blinker	Rz1	A	H
yellow	Blinker	Rz1	A	C
yellow	Blinker	Rz1	A	H
yellow	Blinker	Rz1	A	H
yellow	Blinker	Rz1	A	H
yellow	Blinker	Rz1	A	C
yellow	Blinker	Rz1	A	C
yellow	Blinker	Rz1	A	C
yellow	Blinker	Rz1	A & V	C
yellow	Blinker	Rz1	A	F
yellow	Blinker	Rz1	A	F
yellow	Blinker	Rz1	V	C
yellow	Blinker	Rz1	V	C
yellow	Blinker	Rz1	V	C

# Amino acid motifs in *Rz1* Cultivars in Minnesota



# Amino acid motifs in *Rz1* Cultivars in Minnesota

BNYVV infected *Rz1* blinker plants





# Conclusions

- Predominant motif in *Rz1* diseased spots is  $V_{67}C_{68}$
- This is not the case in *Rz1* blinker plants where there is much more variation in the haplotypes present

# Hypothesis

- Fitness of viral haplotypes
- In time, blinkers will initiate spots of disease in the field as the  $V_{67}C_{68}$  haplotype becomes fixed in the population
- The  $V_{67}C_{68}$  haplotype 'wins' out as the fittest viral strain overcoming *Rz1* resistance
- Not all haplotypes are equally fit and therefore do not initiate diseased patches

# Final thoughts!



# Final Thoughts

- Other mutations present in other parts of the genome? (Tamada *et al.* 2011)
- Influence of minor effect QTL loci in different cultivars on R phenotype

- Is this pattern true of cultivars containing other R sources?
- **'Industry cooperation is key'!**

# Acknowledgements

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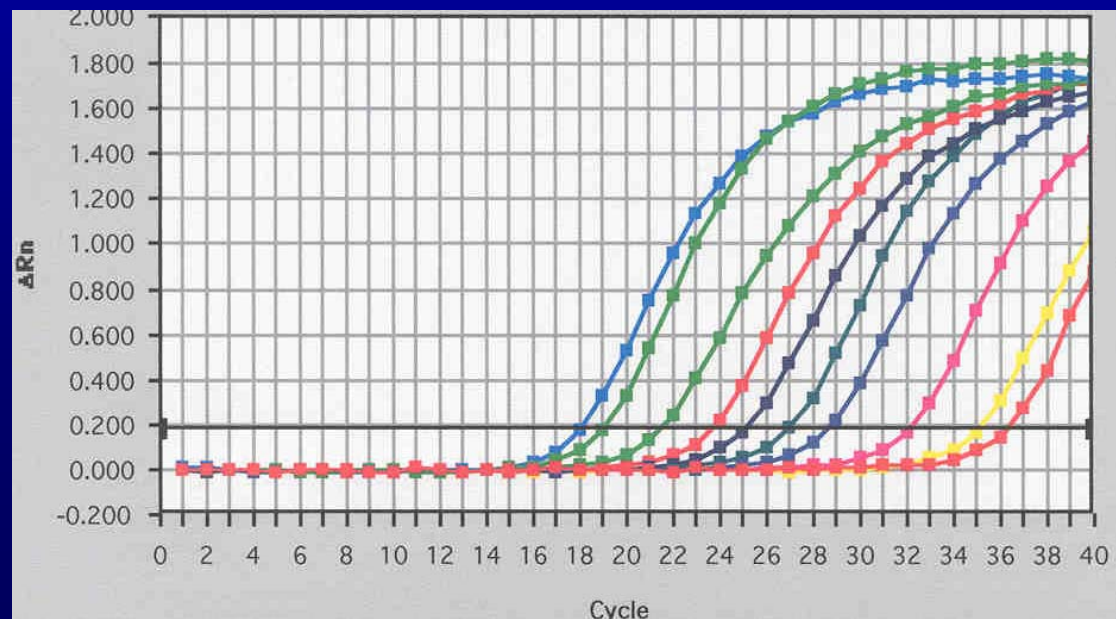


Thank you for listening!



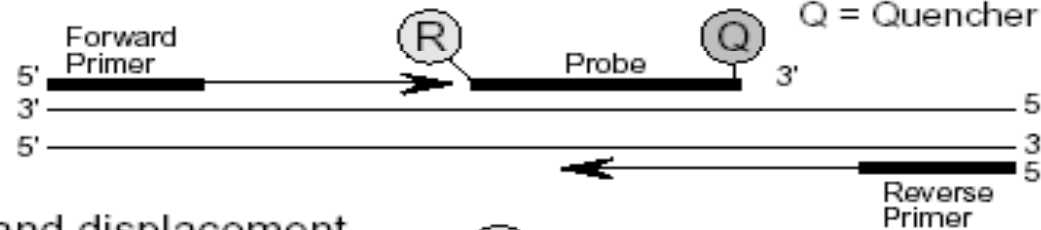
# Real-time PCR

- PCR products are detected as they accumulate
  - DNA binding dyes e.g. SYBR green or specific fluorescent probes
- Amount of DNA amplified is measured after each cycle
- Initial amount of target can be related to cycle threshold (Ct)
- Target DNA quantified using calibration curve relating Ct to known template amounts



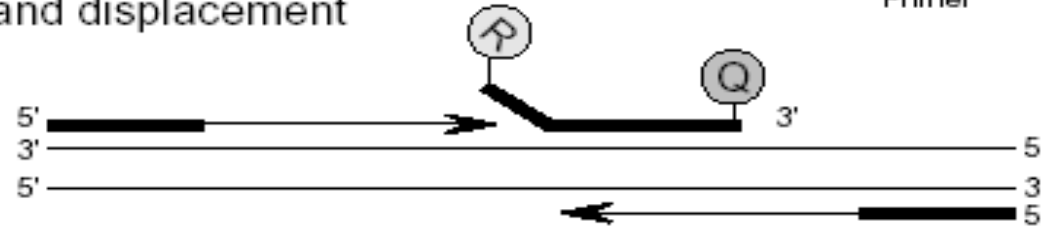
# Real-time PCR

Polymerization



R = Reporter  
Q = Quencher

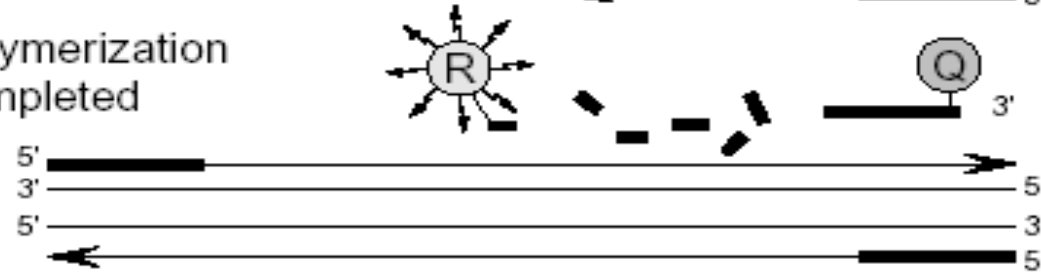
Strand displacement



Cleavage



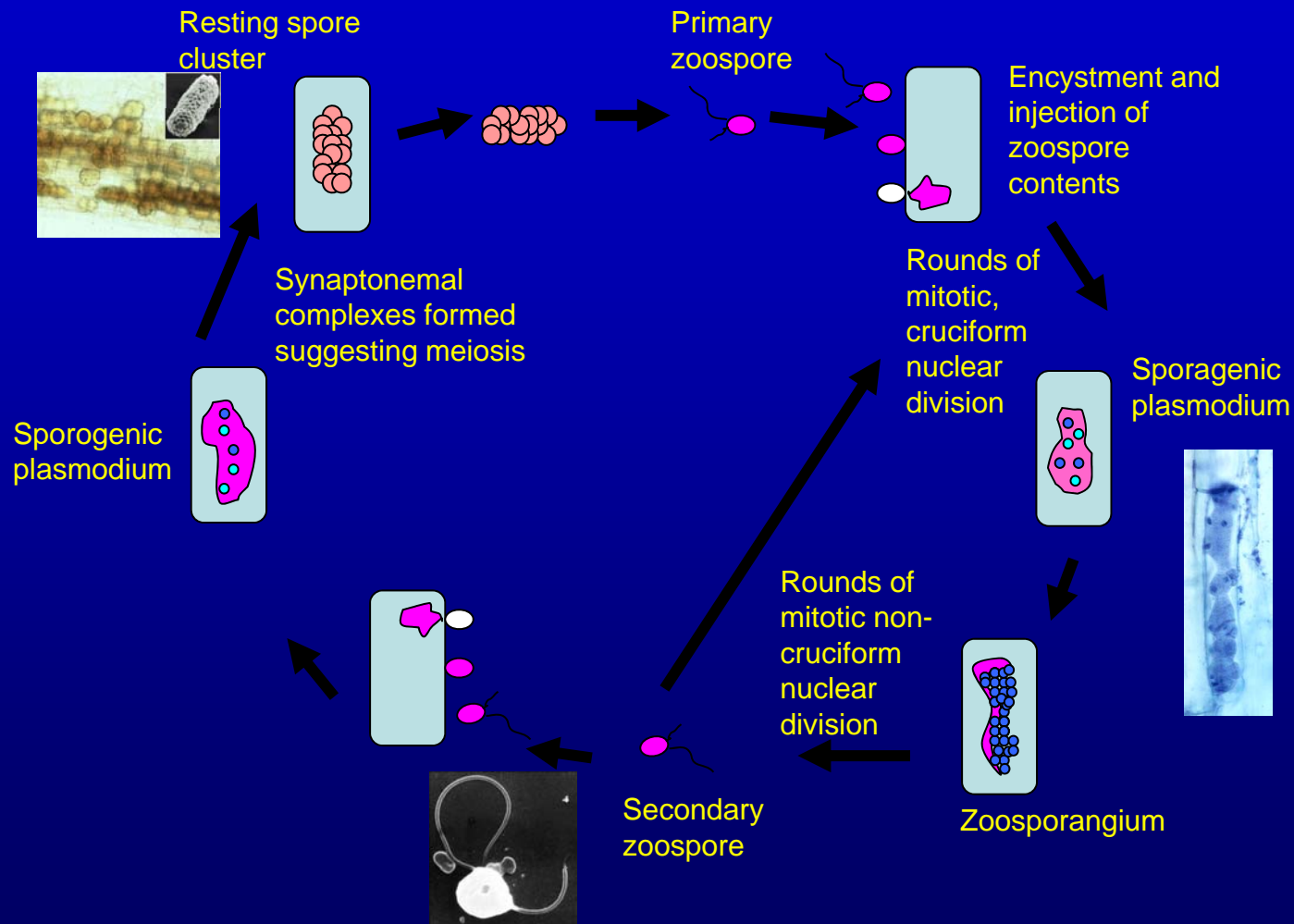
Polymerization completed



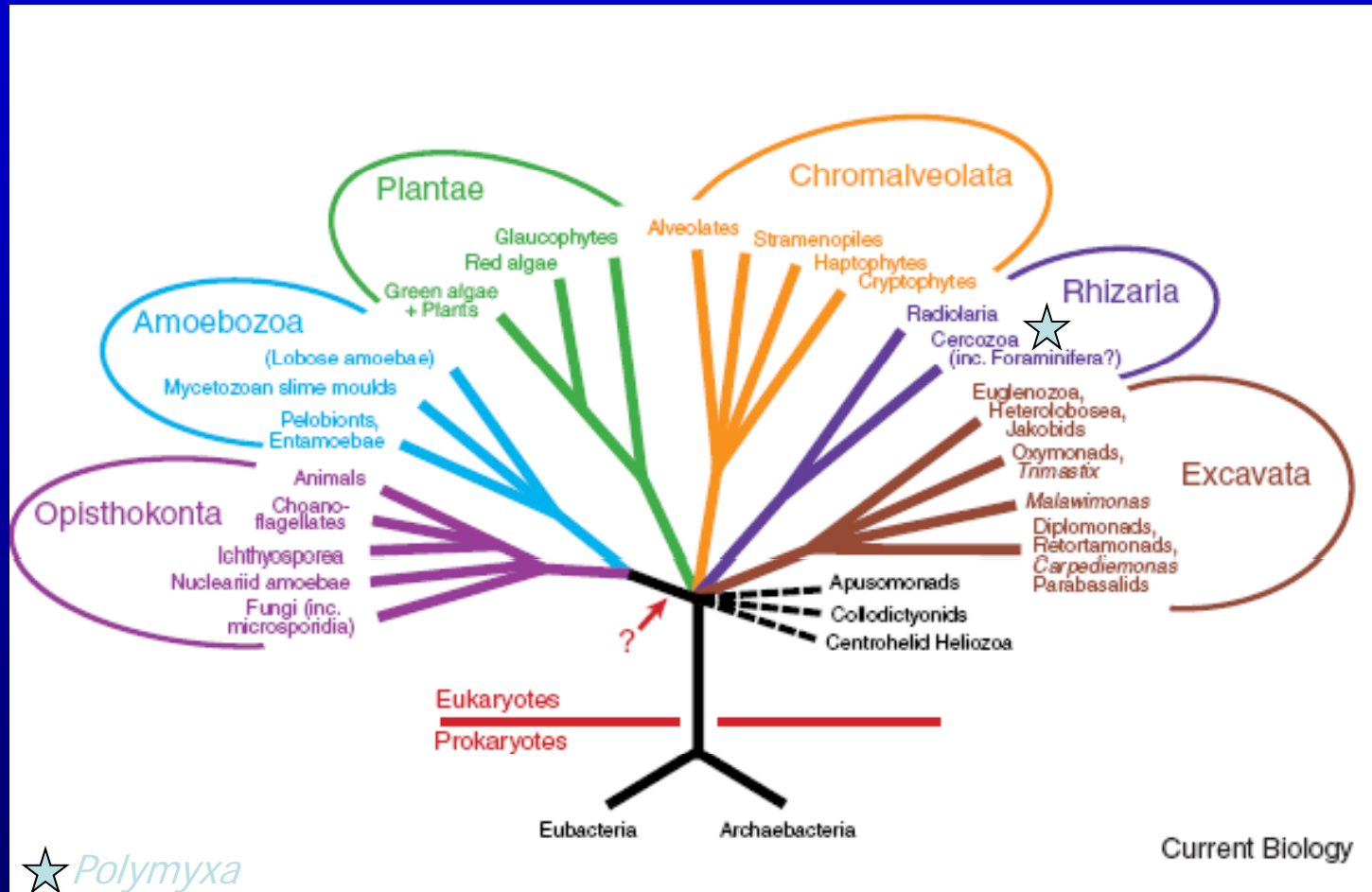
# *What is Polymyxa?*

- Obligate intracellular root-infecting organism
- *Polymyxa* is one of 10 genera in the *Plasmodiophoraceae*
- Two Species: *Polymyxa graminis*  
*Polymyxa betae*

# The Life Cycle of *Polymyxa*



# The Tree of Life



Adl, S.M. et al. (2005) *J. Eukaryot. Microbiol.* 52:399-451

Simpson AG, Roger AJ (2004) *Curr. Biol.* 14:R693-696.