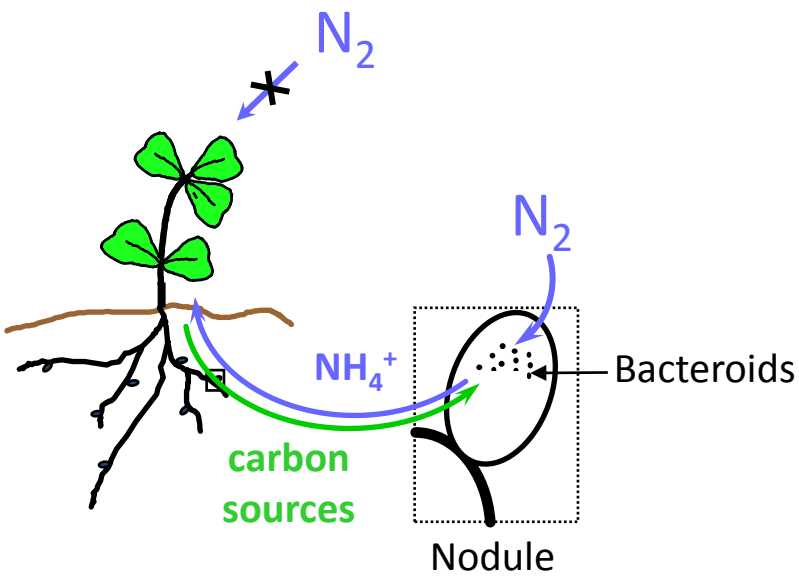
Experimental evolution of rhizobia:
Transient hypermutagenesis accelerates the evolution of legume symbionts following horizontal gene transfer

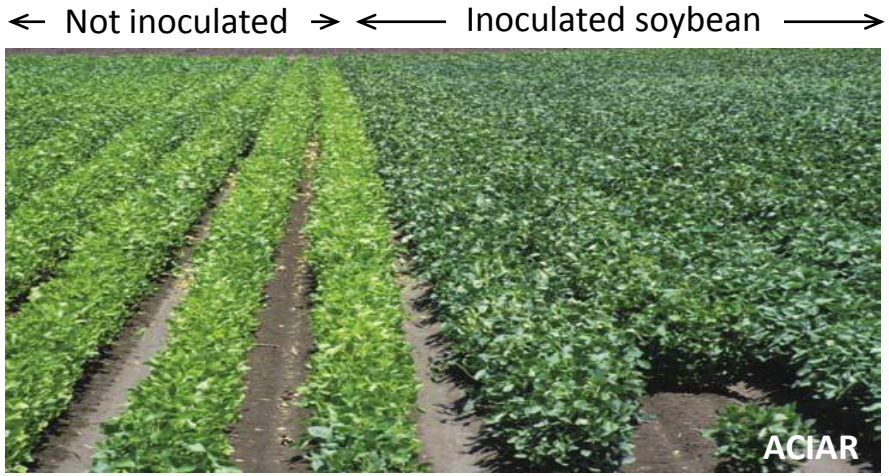
Delphine Capela

Group of Catherine Masson and Jacques Batut

Microbial Bioinformatics - Institut Pasteur - September 25th, 2014

The rhizobium-legume symbiosis, an agronomical and ecological important association





Biological nitrogen fixation by legumes accounts for ~ 100 millions MT of N_2 reduced into NH_4^+

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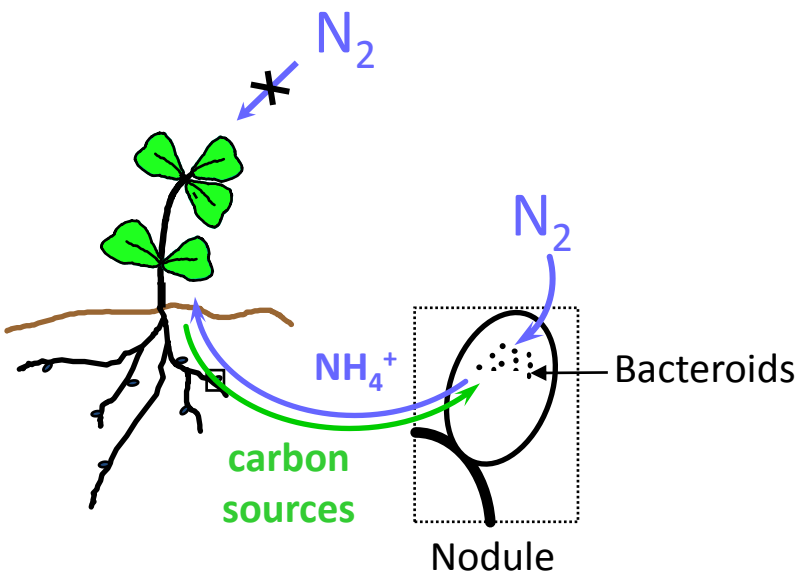


Biological nitrogen fixation by legumes accounts for ~ 100 millions MT of N_2 reduced into NH_4^+

Legumes consist in ~17 000 species

← Not inoculated → ← Inoculated soybean →

Mimosa pudica
C. taiwanensis

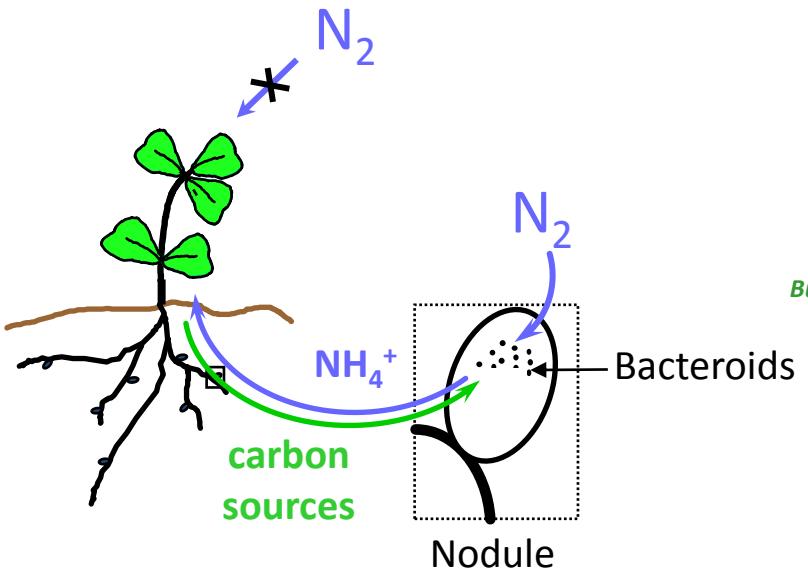
Sesbania rostrata
A. caulinaudans

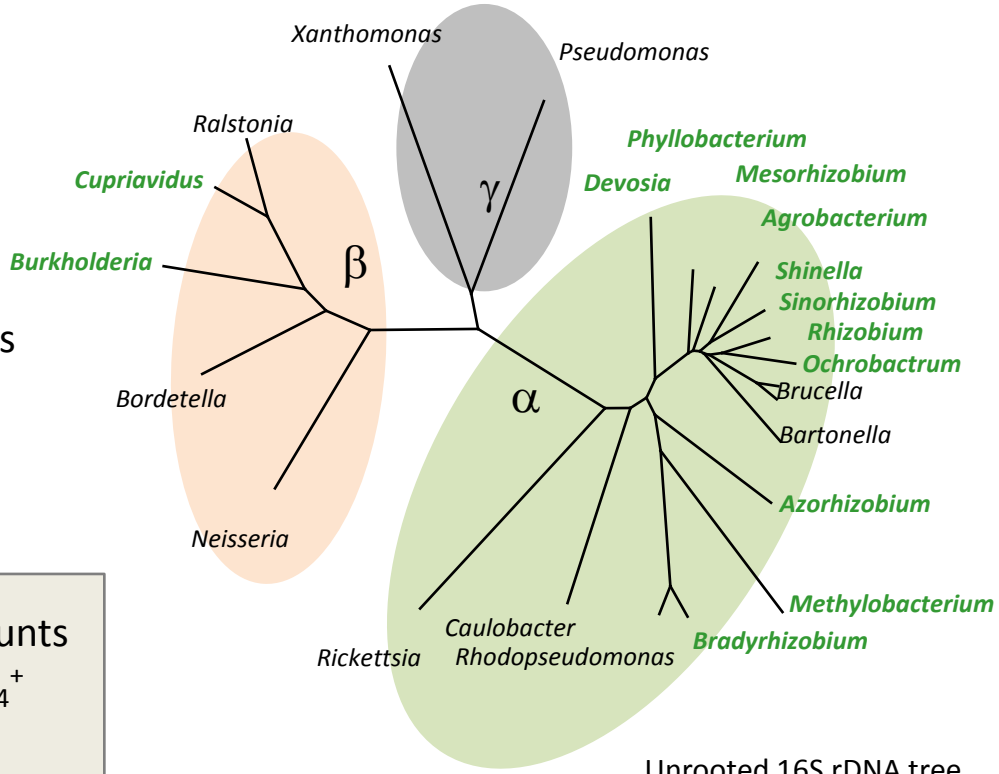
Aeschynomene afraspera
B. sp. ORS322

Aeschynomene sensitiva
B. sp. ORS278

Masson-Boivin *et al.*, *Trends Microbiol*. 2009

The rhizobium-legume symbiosis, an agronomical and ecological important association





Unrooted 16S rDNA tree

Moulin et al., Nature 2001

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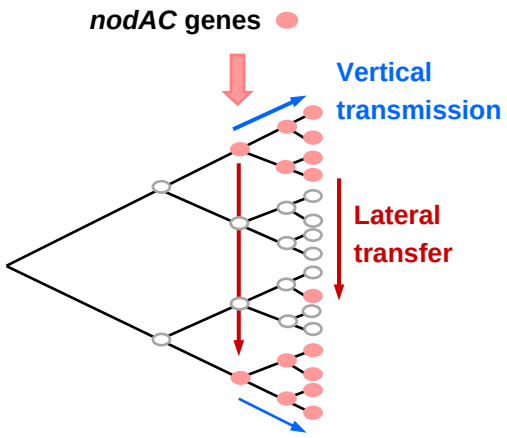
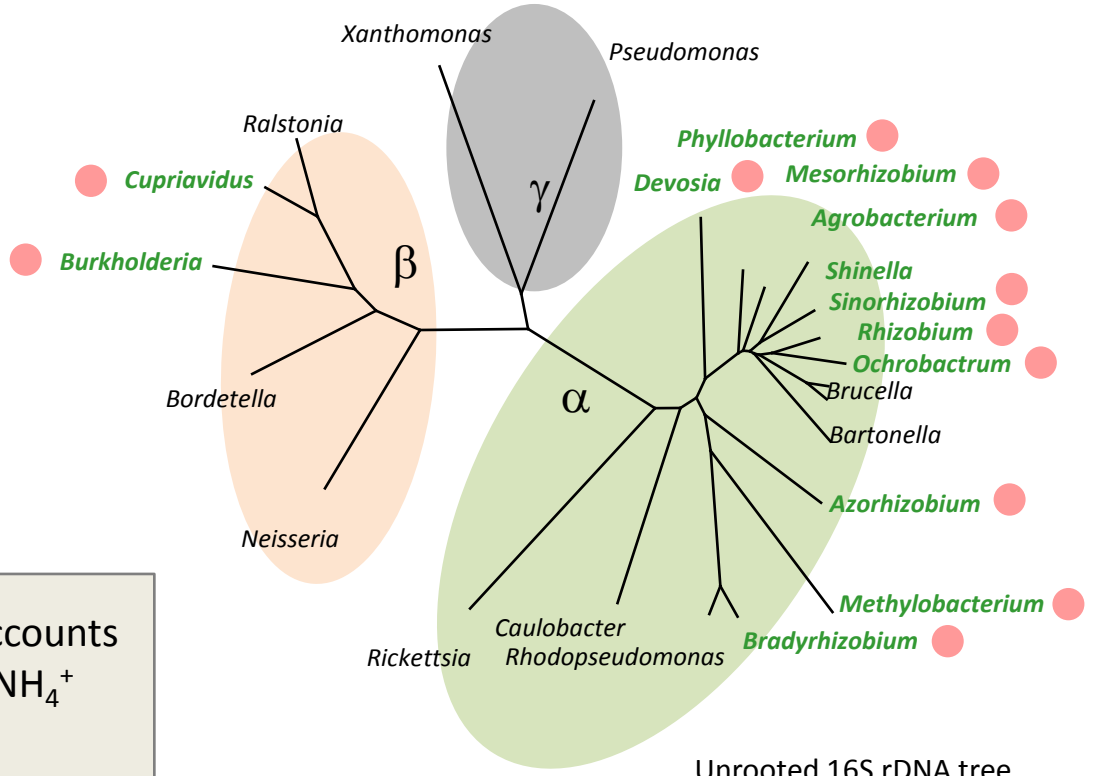
Rhizobia are phylogenetically diverse N_2-fixing legume symbionts





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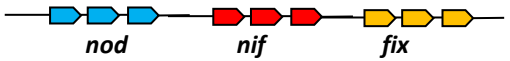
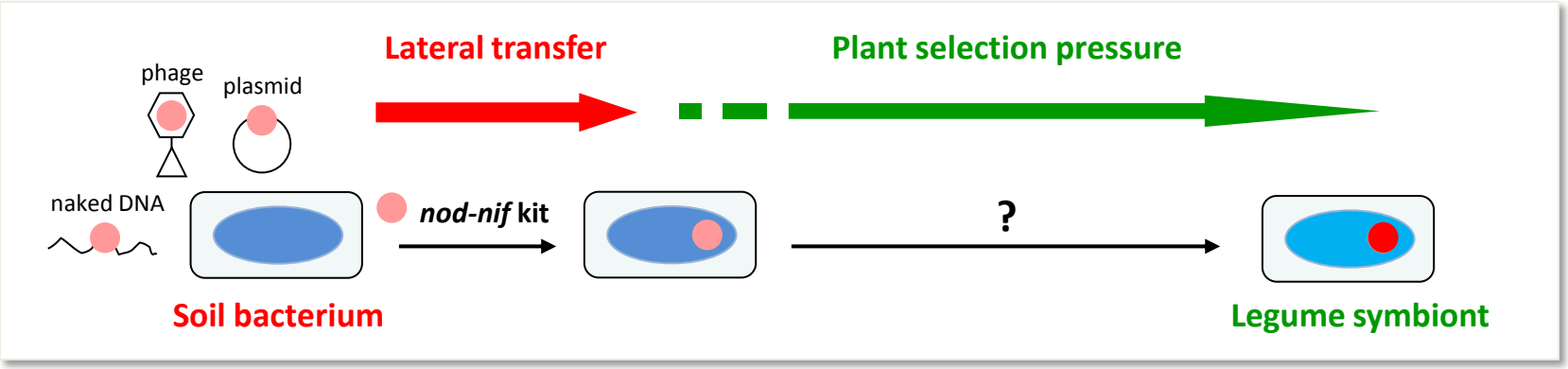
● *nod-nif* kit on MGE



A two step-based scenario for rhizobium evolution



Transfer of key symbiotic genes

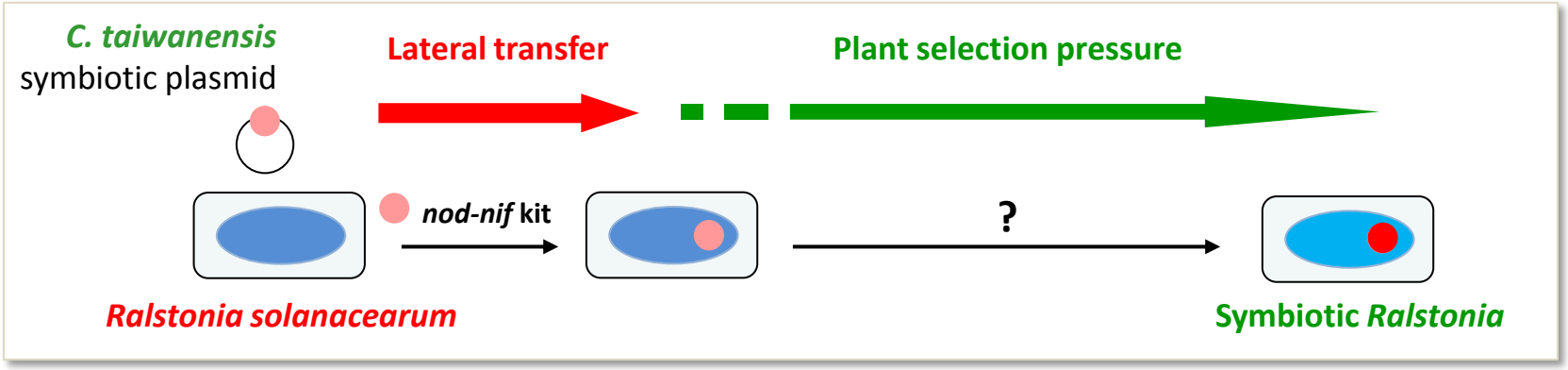
Sullivan *et al ., PNAS* 1995 and 1998

Activation and optimization of the symbiotic potential

Masson-Boivin *et al., TIM* 2009

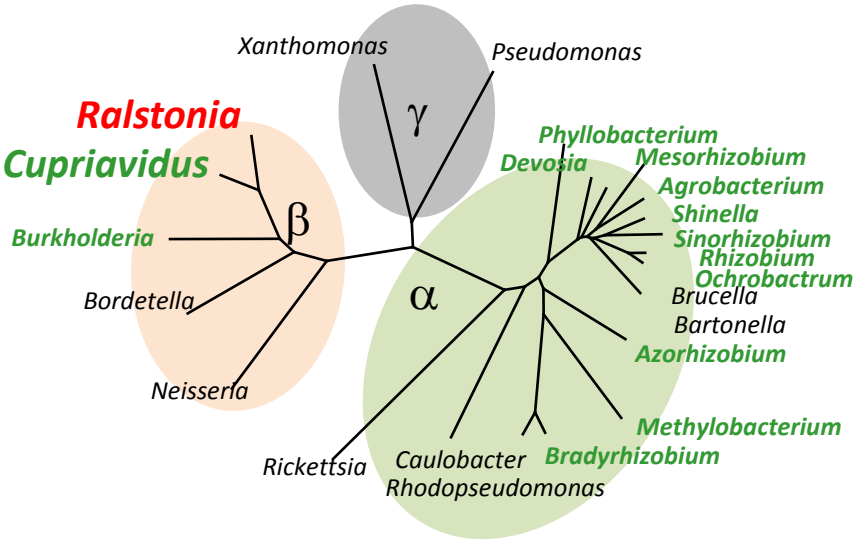
How complex phenotypic traits such as the ability to fix nitrogen with legumes have successfully spread over large phylogenetic distances?

Experimental evolution of a plant pathogen into legume symbionts



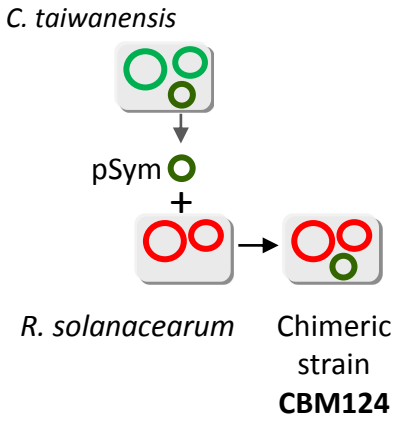
Synthetic biology

Experimental evolution
& whole genome sequencing



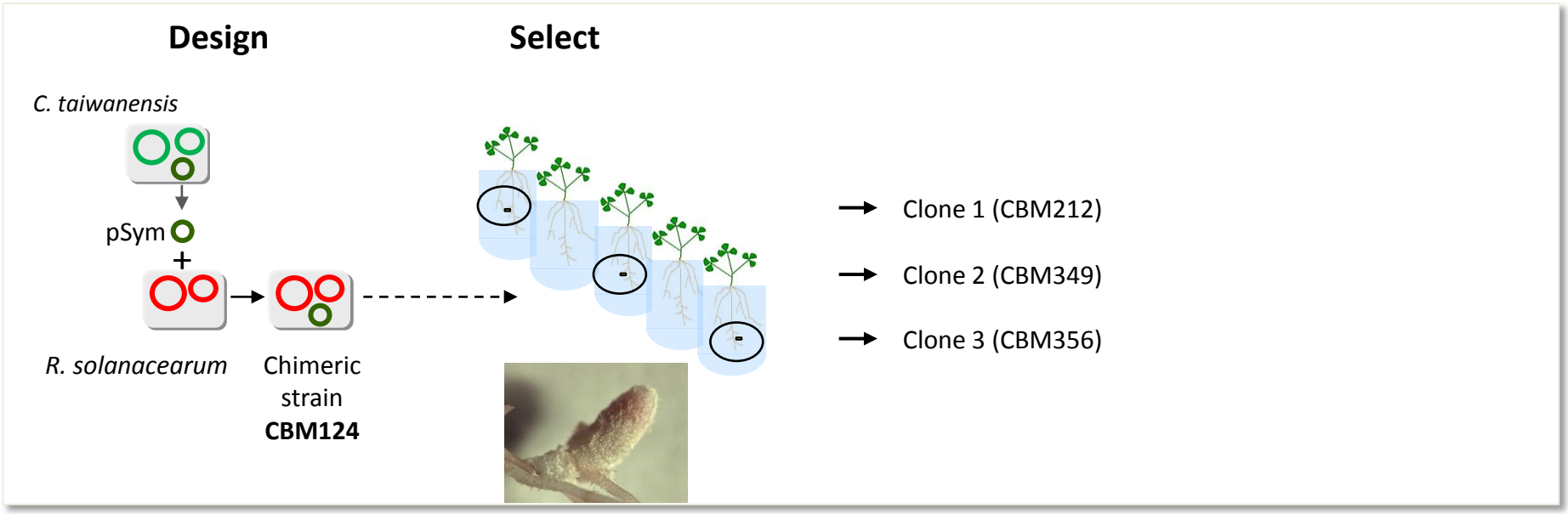
Construction of the chimeric strain

Design



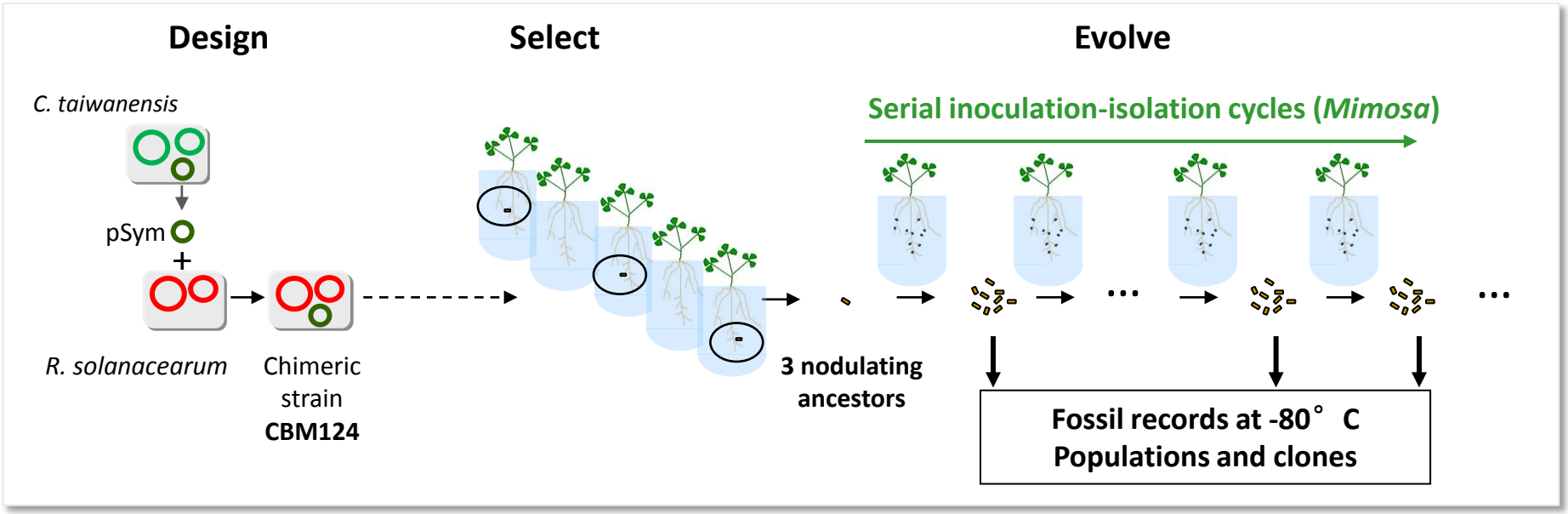
Marchetti *et al*., *PLoS Biol.* 2010

Obtaining nodulating chimeric clones

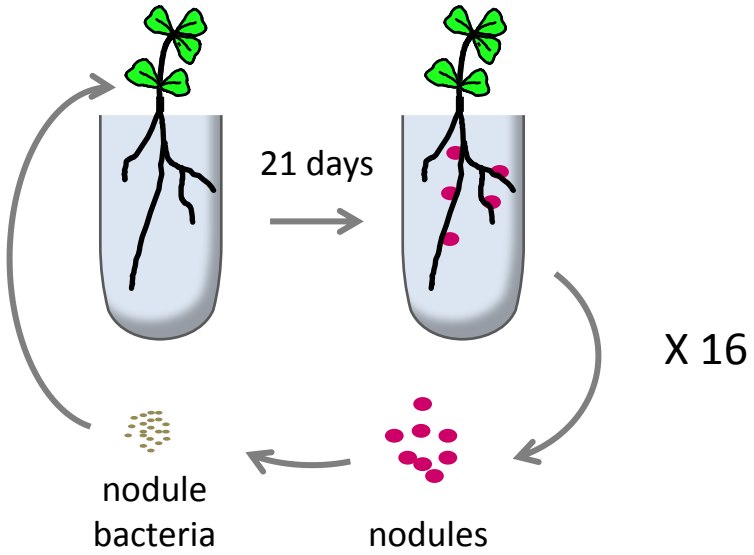


Marchetti *et al*., *PLoS Biol.* 2010

The evolution experiment



Experimental evolution cycles



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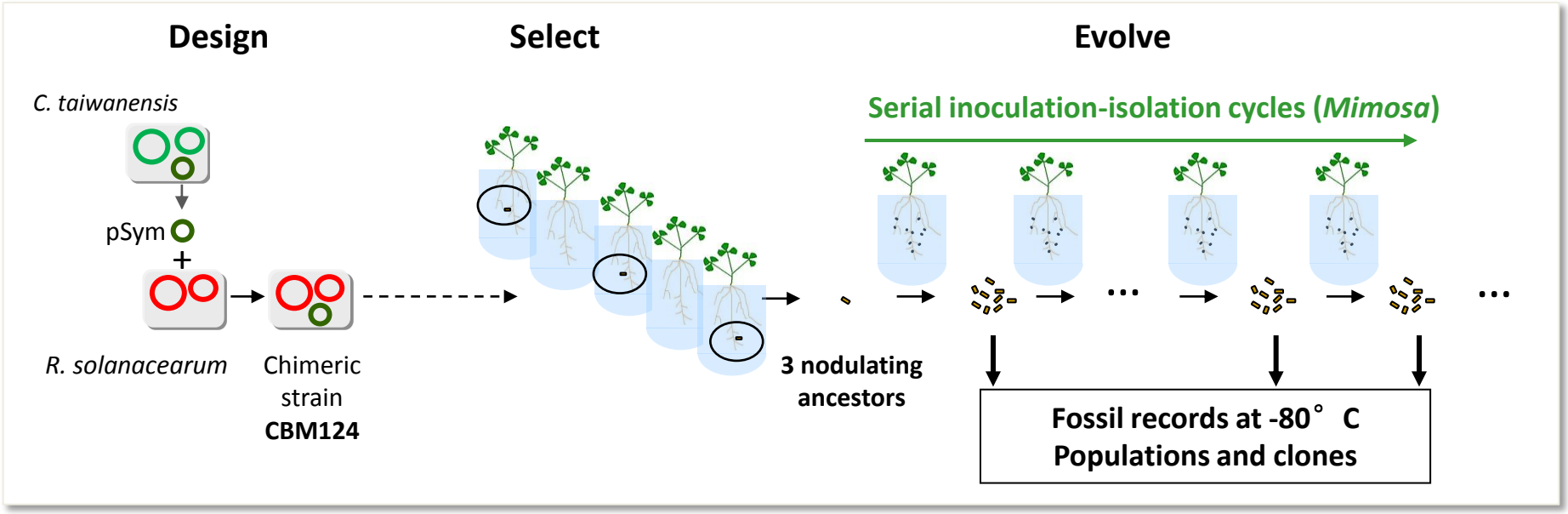
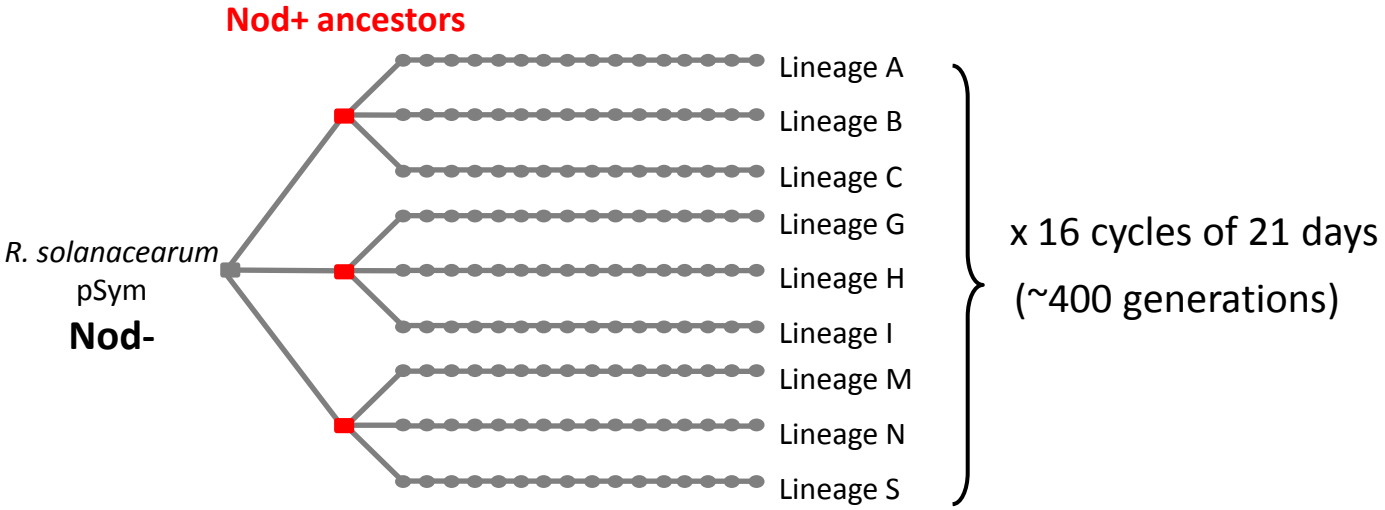
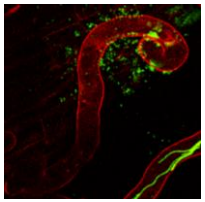
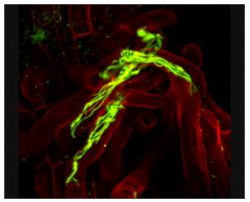




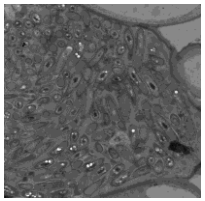
Evolution of symbiotic properties of evolved clones

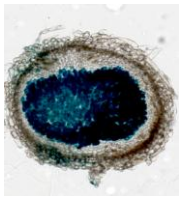
C. taiwanensis / M. pudica


Infection site formation


Infection thread formation


Nodule formation


Intracellular infection


Bacteroid persistence


Nitrogen fixation

Root hair entry and nodulation

Nodule cell infection

N_2 fixation

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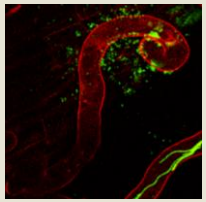
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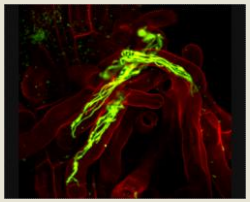
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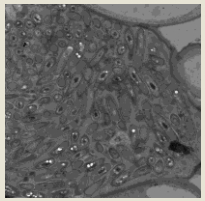
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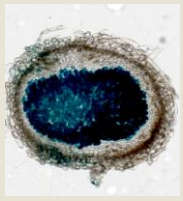
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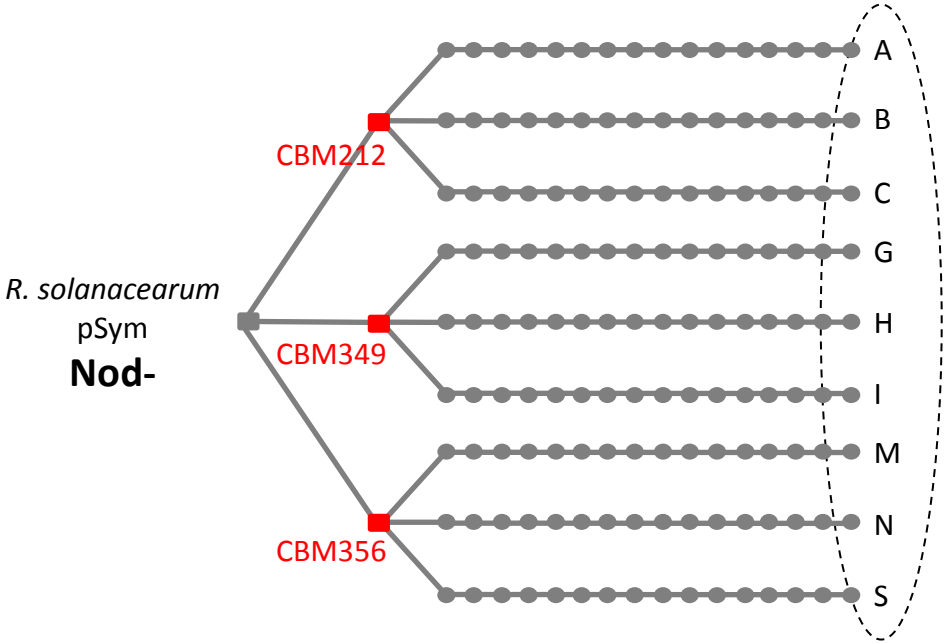
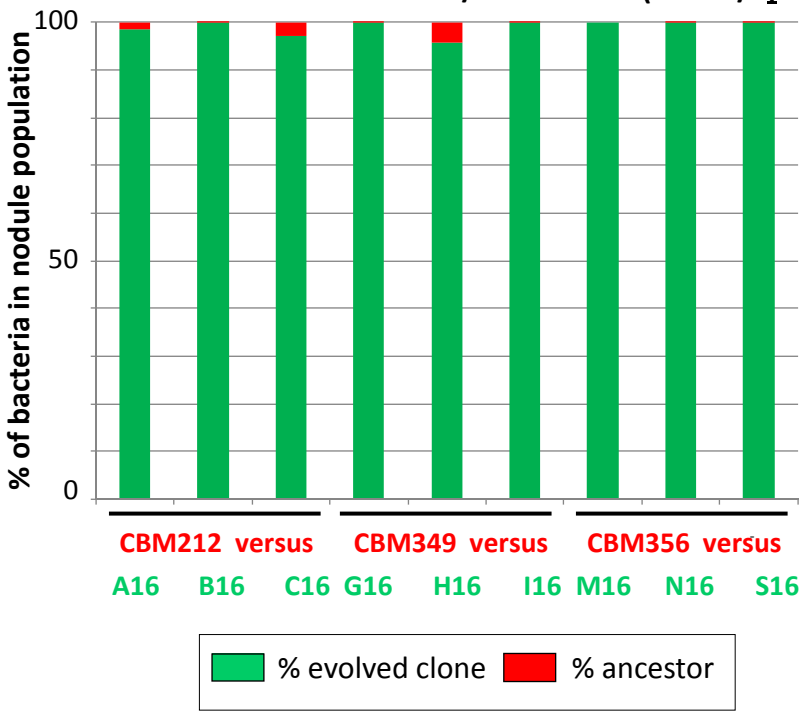
16 cycles, 400 generations



***In planta* relative fitness**

Co-inoculations of ancestor / final clones (ratio 1/1)



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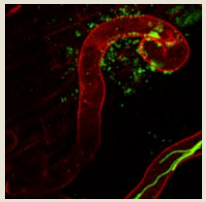
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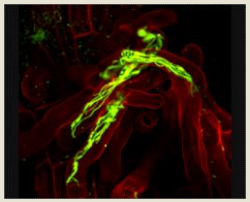
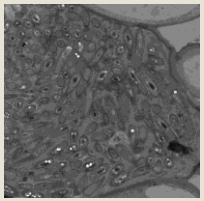

Infection thread formation


Nodule formation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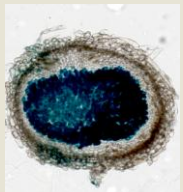
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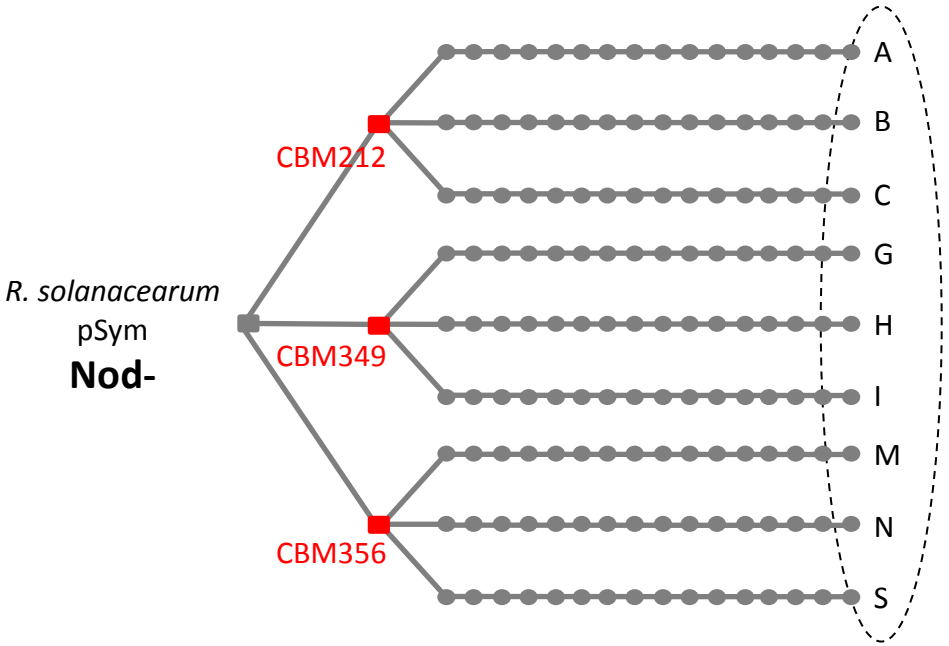

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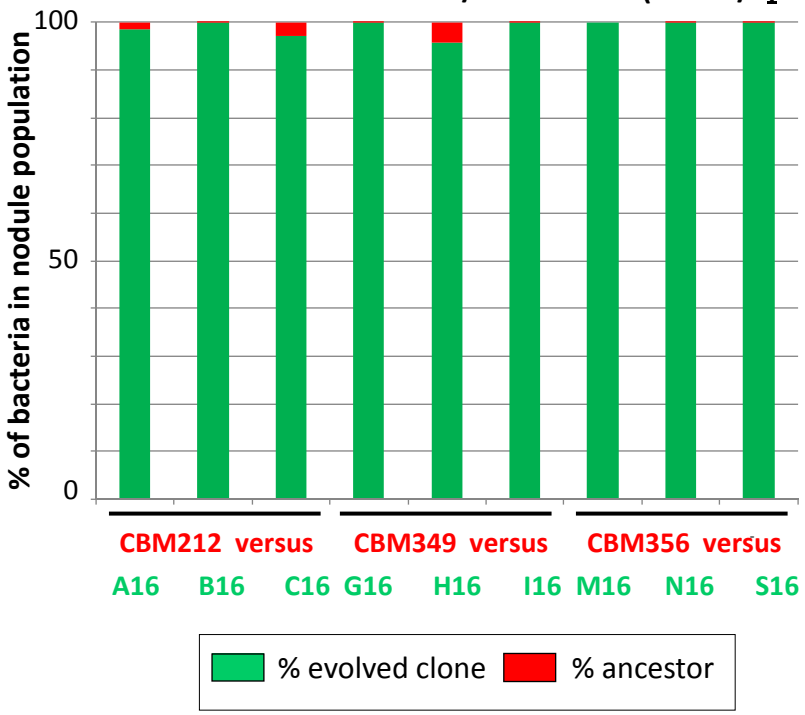
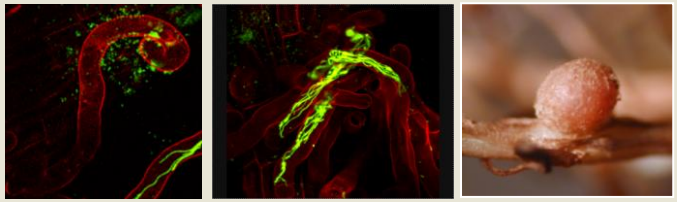


Activation of nodulation

C. taiwanensis / M. pudica



Infection site formation | Infection thread formation | Nodule formation

Root hair entry and nodulation

R. solanacearum
pSym
Nod-

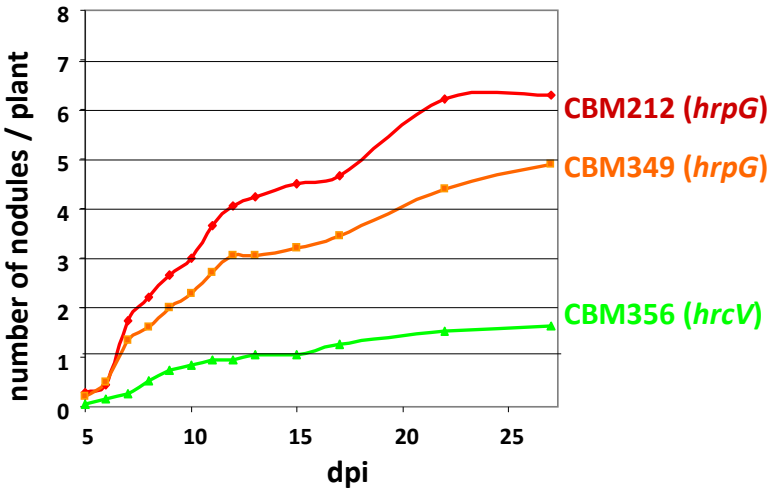
CBM212
Nod+
40 mut, *hrpG**

CBM349
Nod+
29 mut, *hrpG**

CBM356
Nod+/-
19 mut, *hrcV**

SNPs/Indels
(SNiPer - MaGe MicroScope platform)

Nodulation kinetics



Marchetti *et al.*, *PLoS Biol.* 2010

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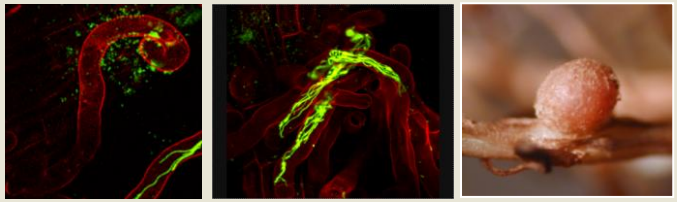

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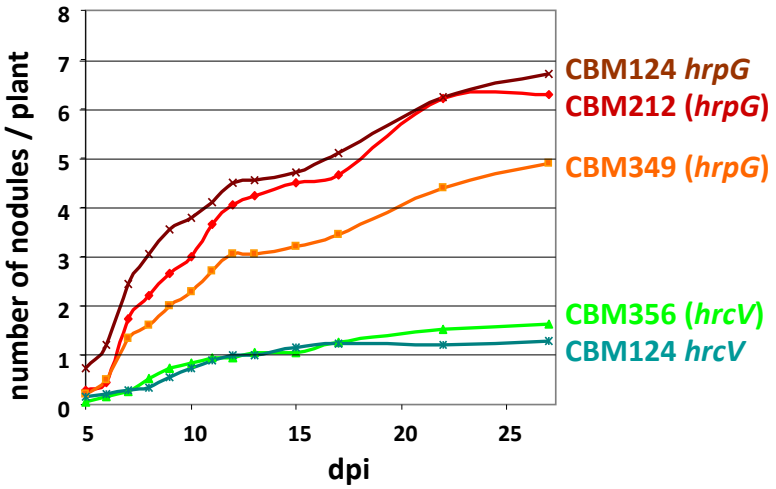

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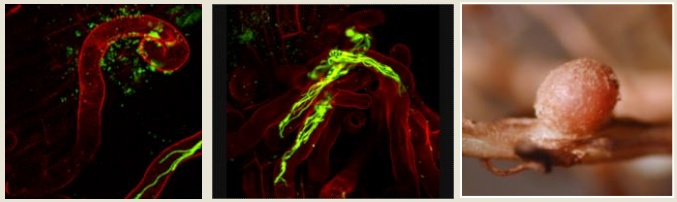

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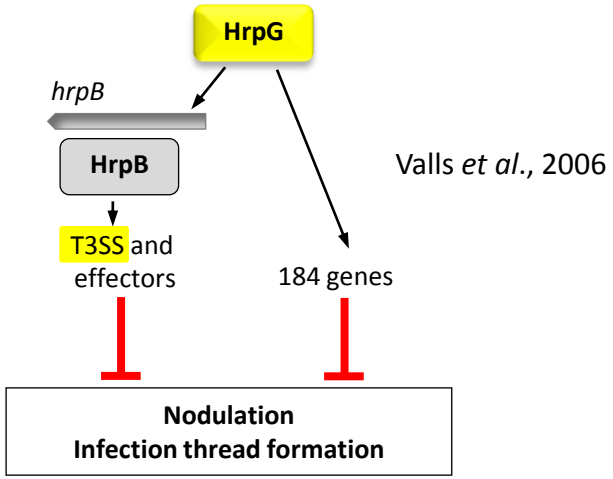
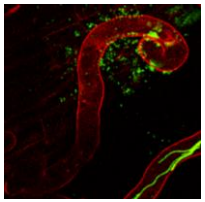


Valls *et al.*, 2006

Marchetti *et al.*, *PLoS Biol.* 2010

Activation of intracellular infection

C. taiwanensis / M. pudica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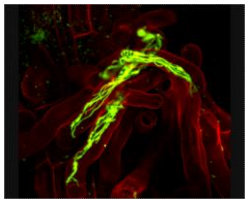
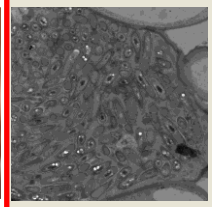
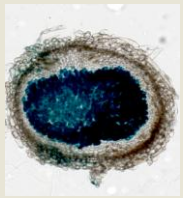

Infection thread formation


Nodule formation


Intracellular infection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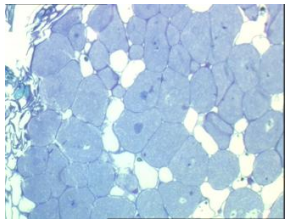
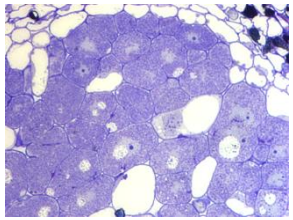
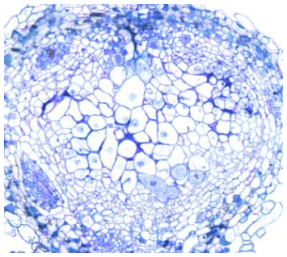
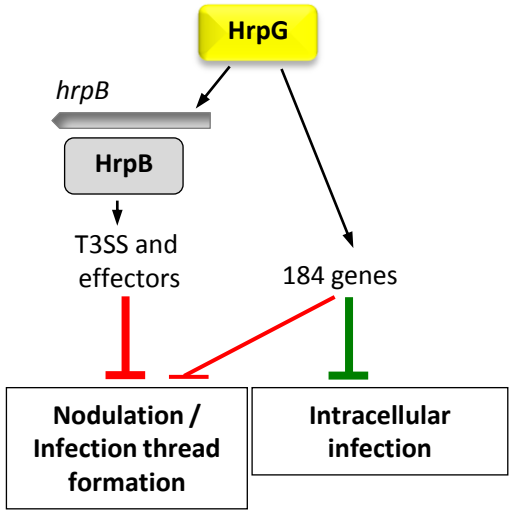






*hrpG**

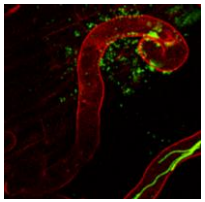
*hrcV**



Marchetti *et al.*, PLoS Biol. 2010

Activation of intracellular infection

C. taiwanensis / M. pudica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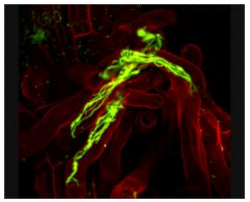
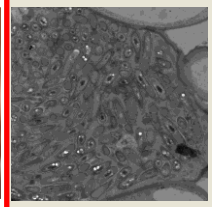
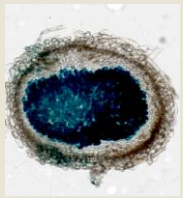

Infection thread formation


Nodule formation


Intracellular infection


Bacteroid persistence

Root hair entry and nodulation

Nodule cell infection

Activation of nodulation

Activation of infection

16 cycles, 400 generations

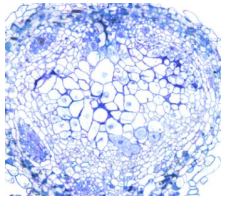
R. solanacearum
pSym
Nod-

A
B
C
G
H
I
M
N
S

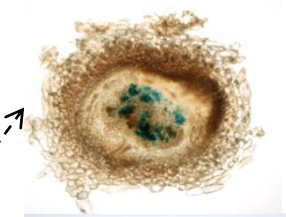
3 mut

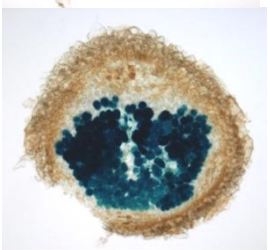
3 mut

2 mut



nodB-lacZ


M16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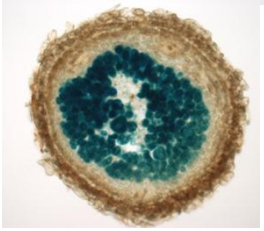
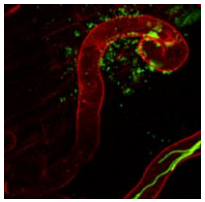
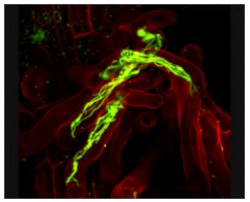

S16

Guan *et al.,* ISME J. 2013

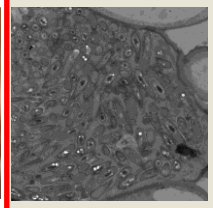
Activation of intracellular infection

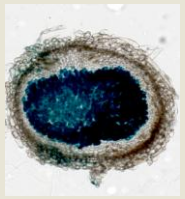
C. taiwanensis / M. pudica


Infection site formation


Infection thread formation


Nodule formation


Intracellular infection


Bacteroid persistence

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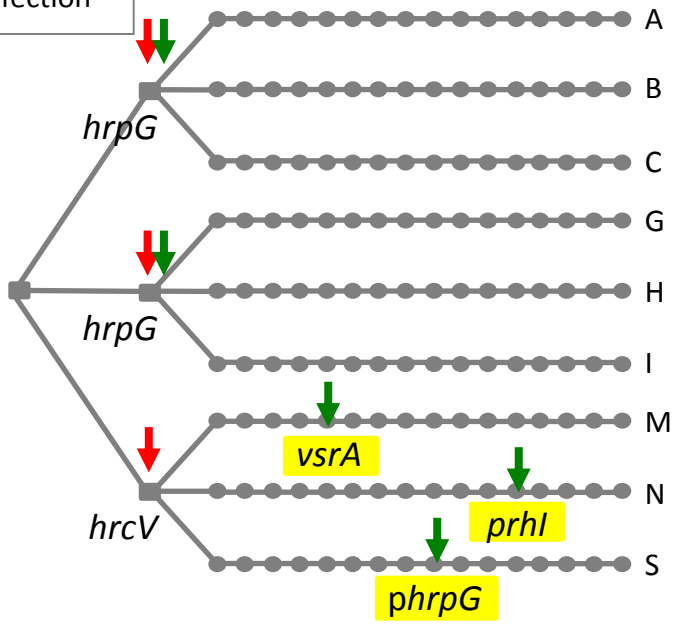
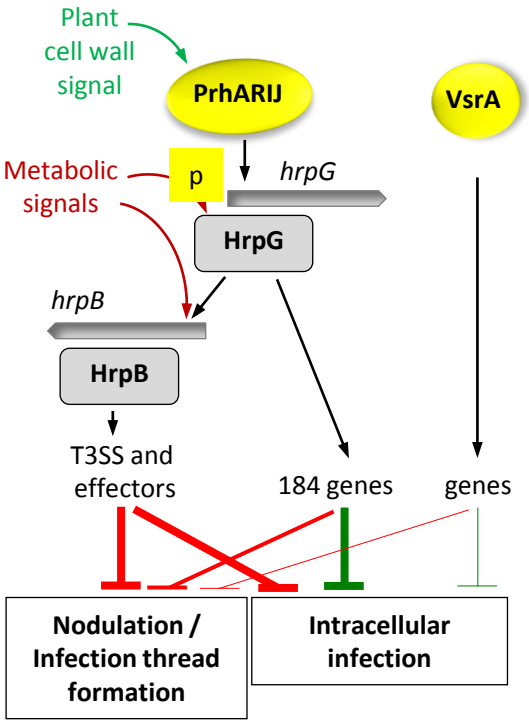
16 cycles, 400 generations

R. solanacearum
pSym
Nod-





Guan *et al.*, ISME J. 2013

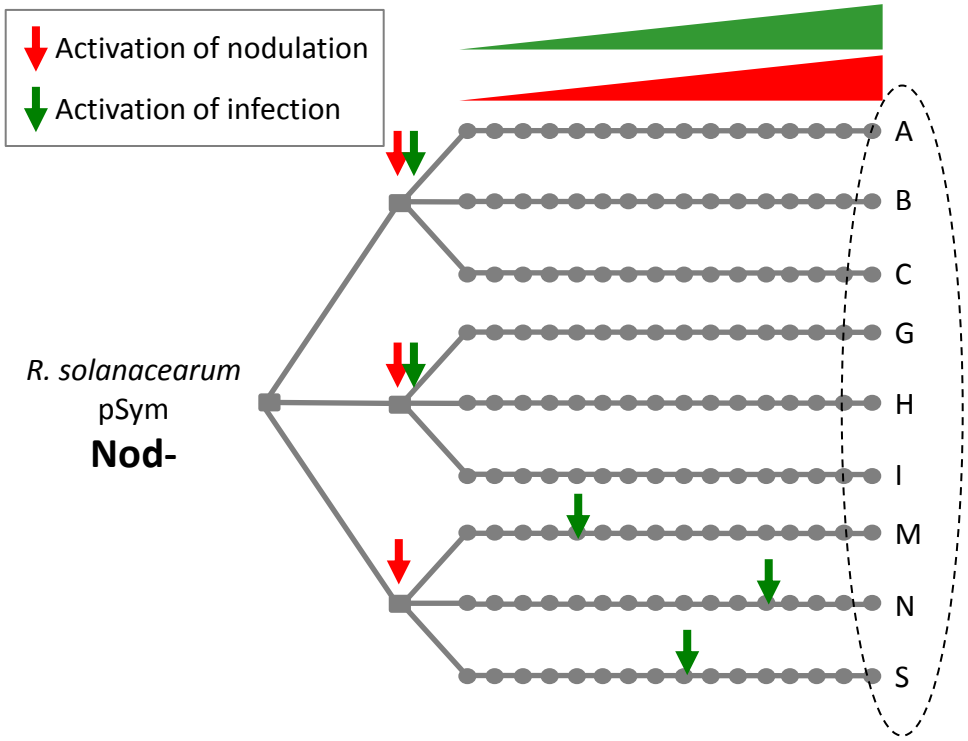
Improvement of nodulation and infection capacities along cycles

C. taiwanensis / M. pudica

Infection site formation | Infection thread formation | Nodule formation | Intracellular infection | Bacteroid persistence

Root hair entry and nodulation

Nodule cell infection



R. solanacearum
pSym
Nod-

↗ Nodulation competitiveness

↗ Intracellular infection

↗ Bacteroid persistence

↙ Defense reactions

Marchetti *et al.,* MPMI 2014

Improvements in symbiotic properties occurred by sharp shifts

C. taiwanensis / M. pudica

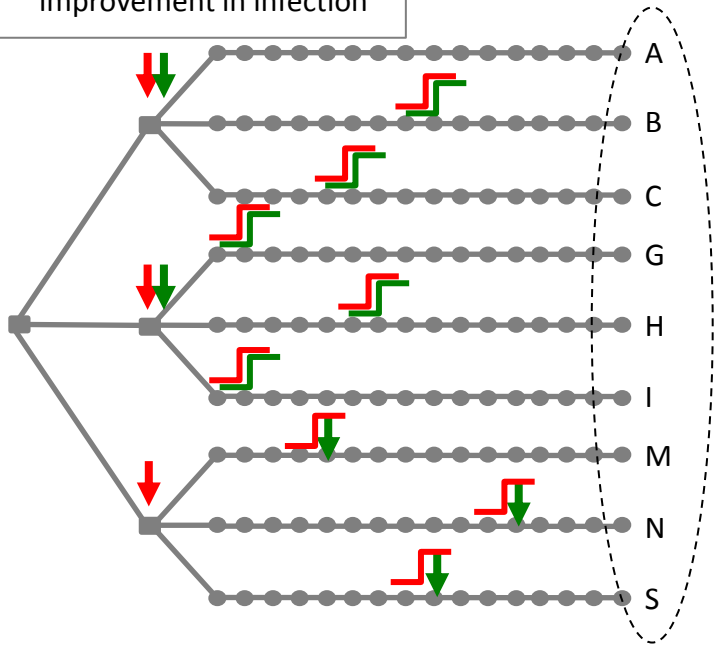
Infection site formation | Infection thread formation | Nodule formation | Intracellular infection | Bacteroid persistence

Root hair entry and nodulation

Nodule cell infection

Activation, improvement in nodulation

Activation , improvement in infection



R. solanacearum
pSym
Nod-

↗ Nodulation competitiveness

↗ Intracellular infection

↗ Bacteroid persistence

↙ Defense reactions

Evolution of symbiotic properties of evolved clones

C. taiwanensis / M. pudica

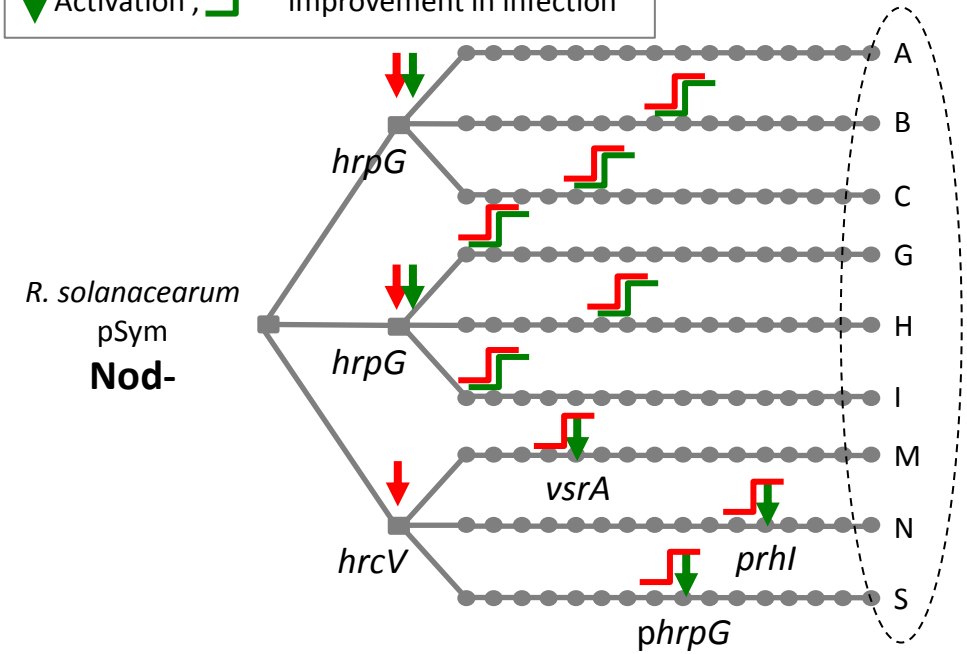
Infection site formation | Infection thread formation | Nodule formation | Intracellular infection | Bacteroid persistence

Root hair entry and nodulation

Nodule cell infection

Activation, improvement in nodulation

Activation , improvement in infection



Evolution is sharp and fast: nodulation and infection were rapidly acquired and strongly improved

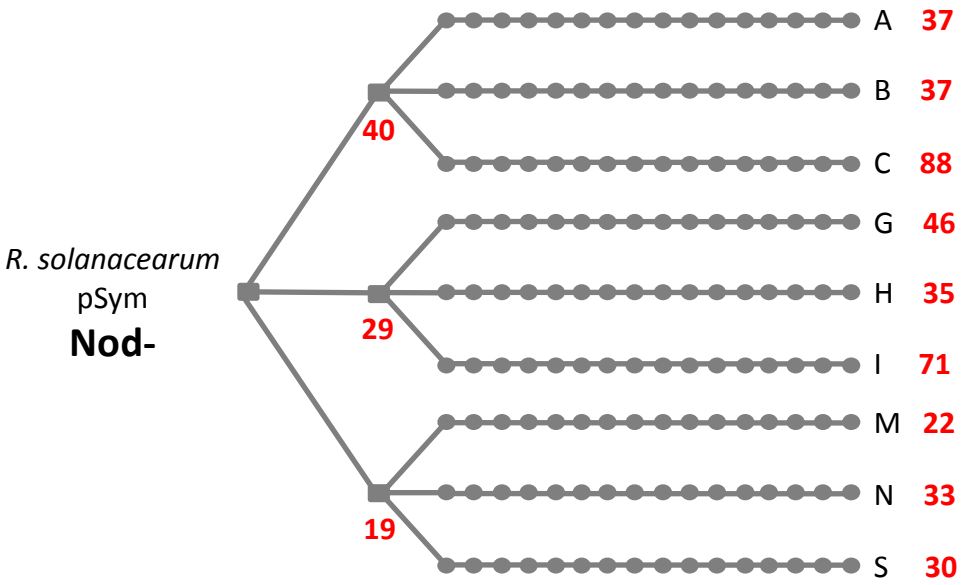
Evolution requires genome remodelling

Antagonism between symbiosis and elicitation of defense reactions

Regulators are preferred targets for evolution

Over-abundance of mutations in evolved clones

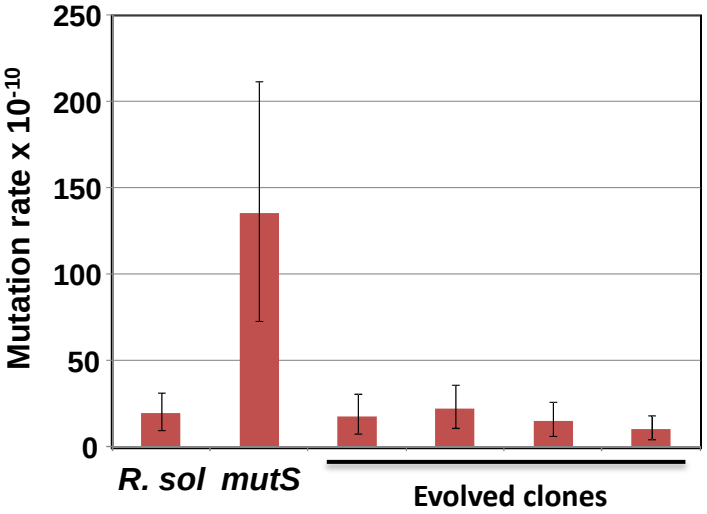
SNPs/Indels (PALOMA - MaGe MicroScope platform)



~ 75 point mutations / clone in 400 generations!

Estimated mutation rate: 3.10^{-8} syn mutations/bp/generation

Fluctuation tests in rich medium



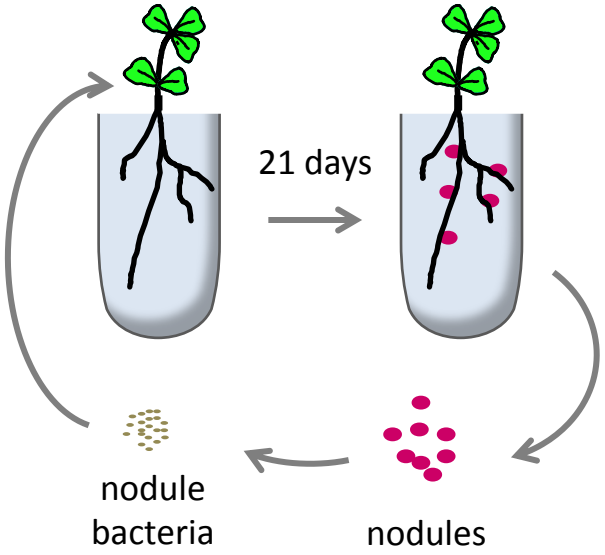
- ✓ **Evolved clones have no mutation in DNA repair systems**
- ✓ **They are not constitutive hypermutators**

Bacteria likely underwent transient hypermutagenesis during the expriment

Remigi *et al.,* PLoS Biol. 2014

Where do bacteria accumulate mutations?

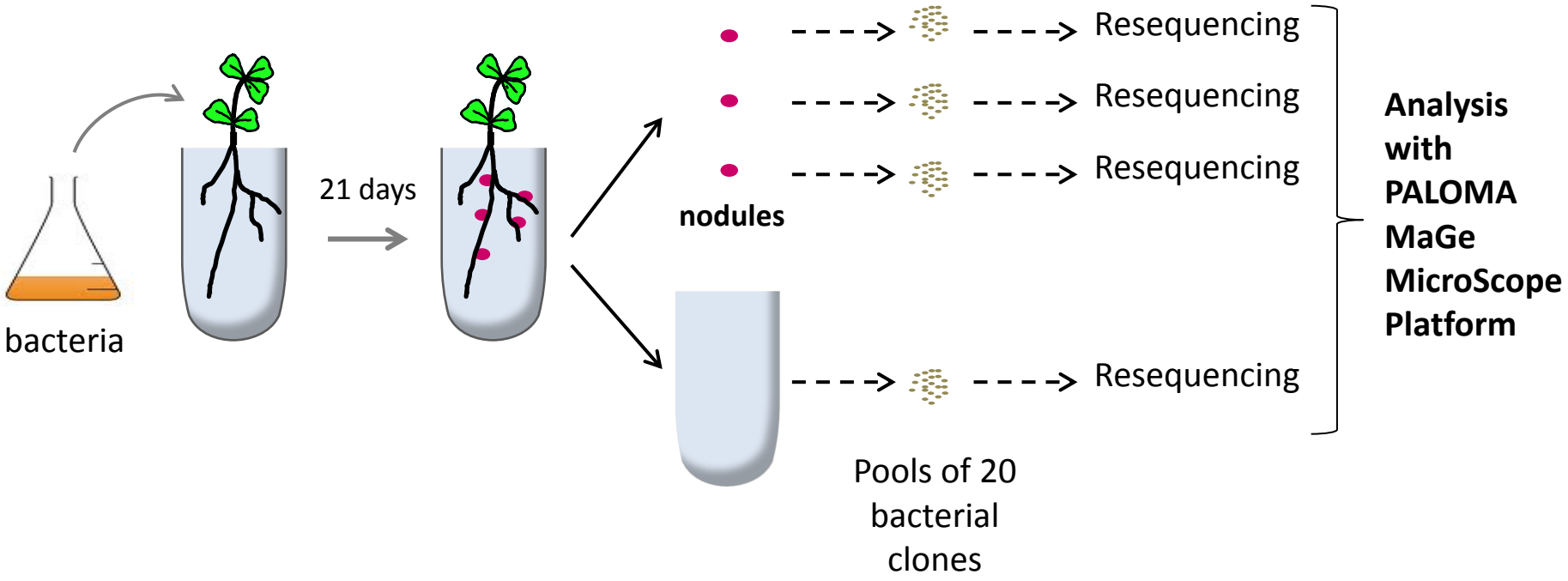
Bacteria were submitted to serial *ex planta* - *in planta* evolution cycle:



1. Colonization of the plant medium (C/N-free medium)
2. Multiplication within nodules

Where do bacteria accumulate mutations?

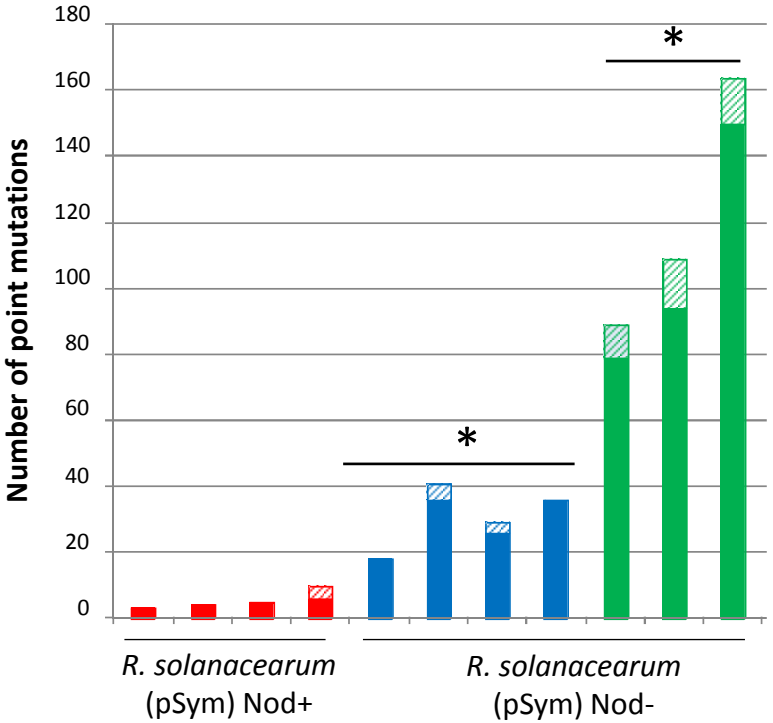
Replaying one cycle of evolution and resequencing populations to analyse the genomic diversity

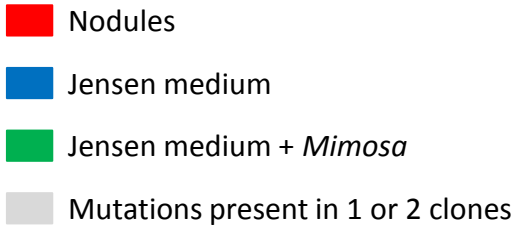


Remigi *et al.,* PLoS Biol. 2014

Transient mutagenesis occurs in the rhizosphere (*ex planta*)

Number of detected SNPs/Indel in pools of 20 clones by whole-genome sequencing

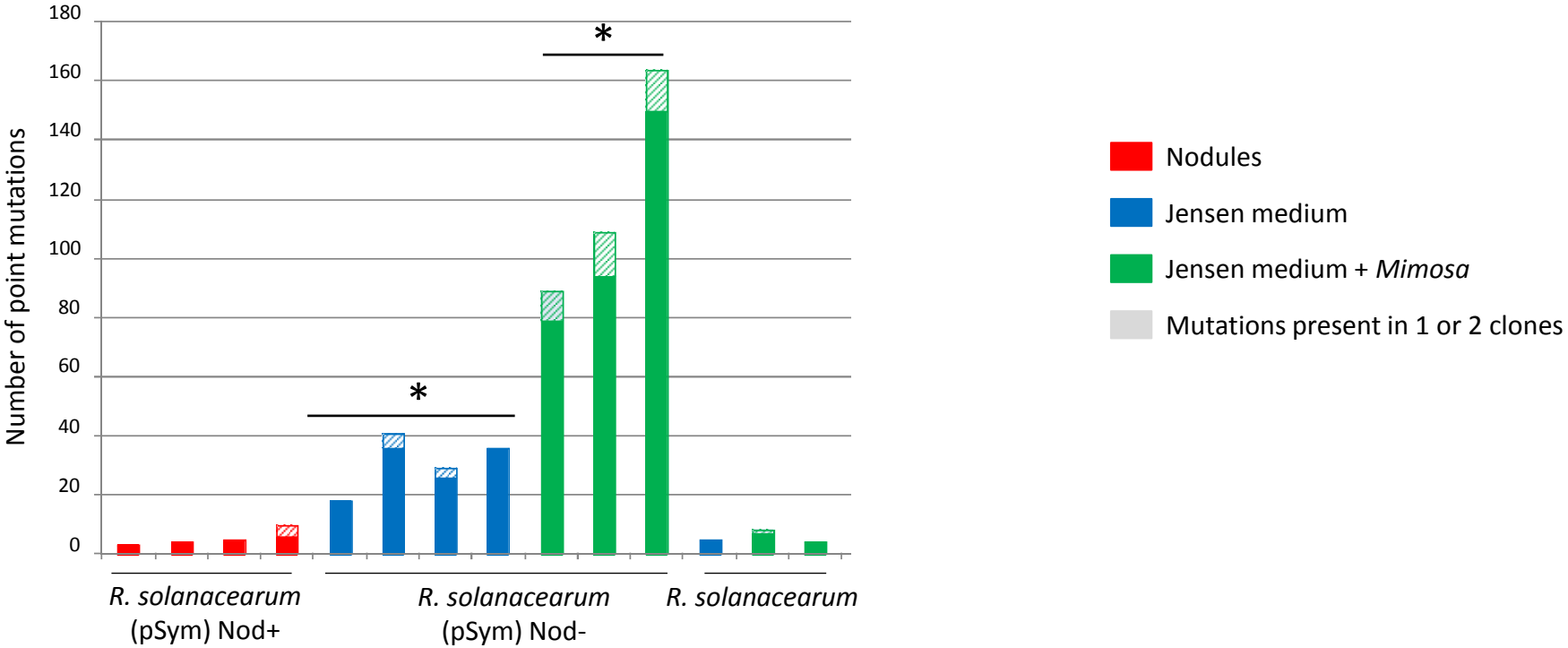




Remigi *et al.,* PLoS Biol. 2014

Transient mutagenesis is dependent on the symbiotic plasmid

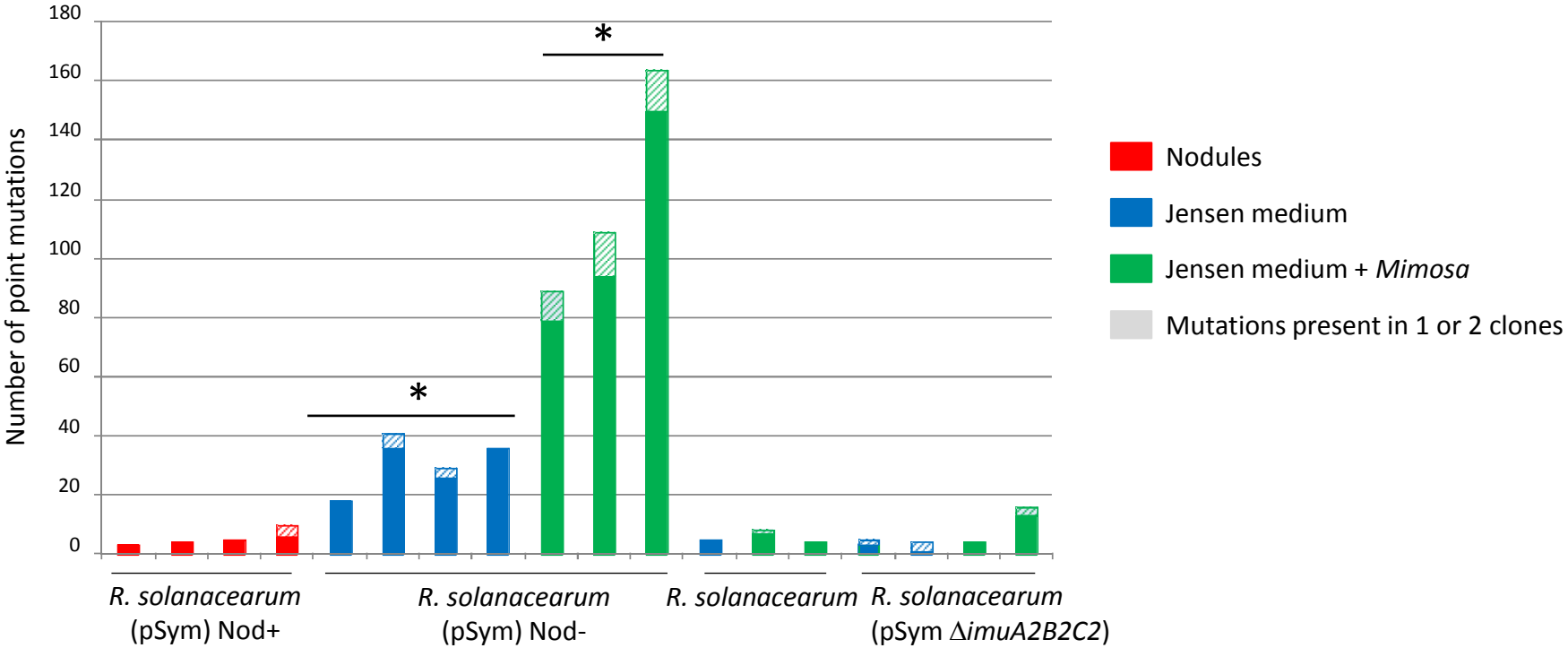
Number of detected SNPs/Indel in pools of 20 clones by whole-genome sequencing



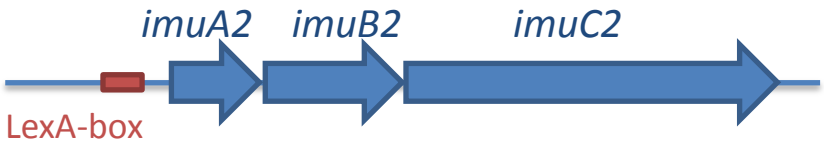
Remigi *et al.,* PLoS Biol. 2014

Plasmid error-prone DNA polymerases trigger hypermutability of the recipient genome

Number of detected SNPs/Indel in pools of 20 clones by whole-genome sequencing



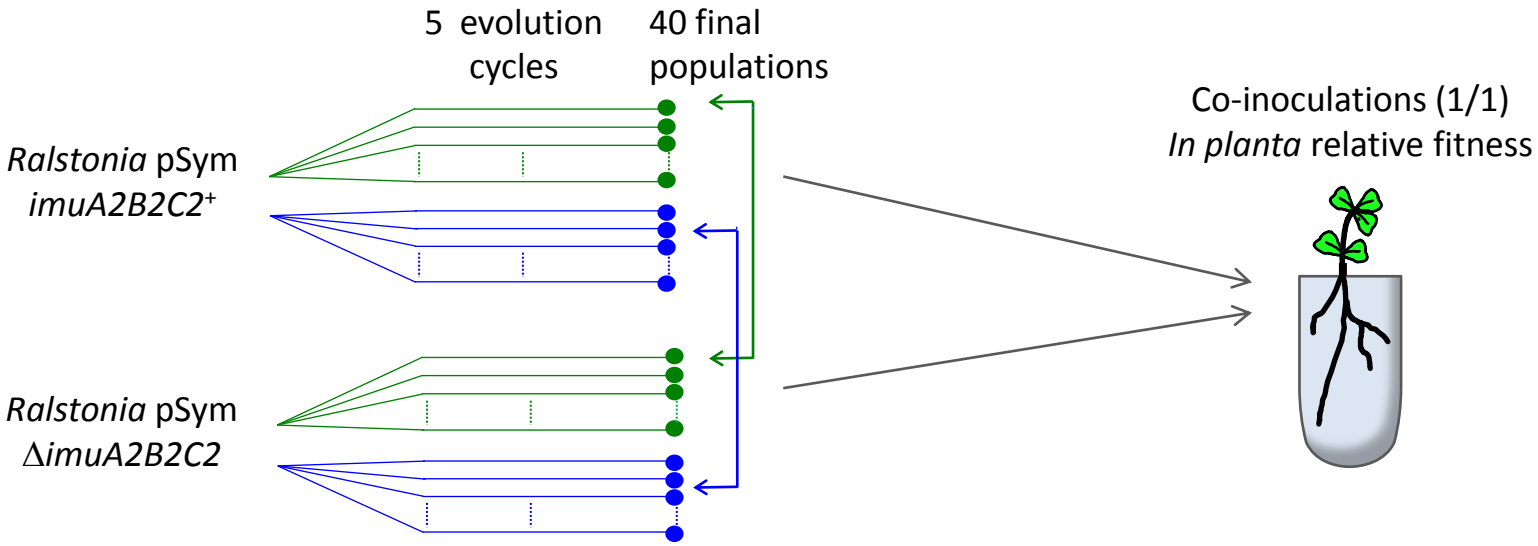
Mutagenesis cassette:



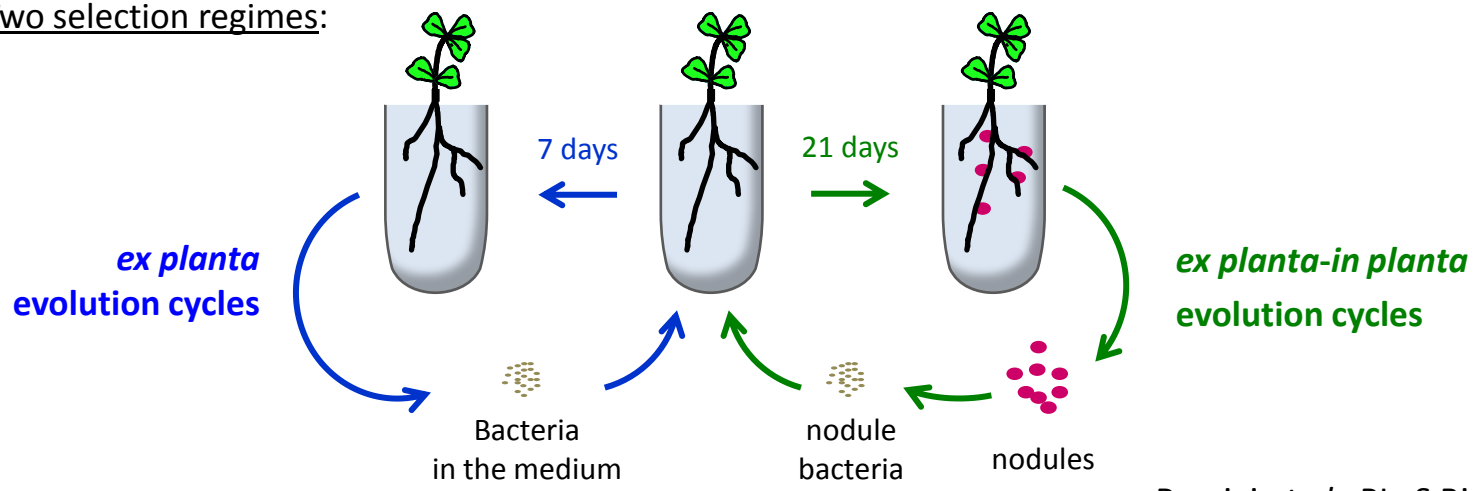
Error-prone DNA polymerase

Remigi *et al.,* PLoS Biol. 2014

How hypermutability impact on symbiotic evolution?

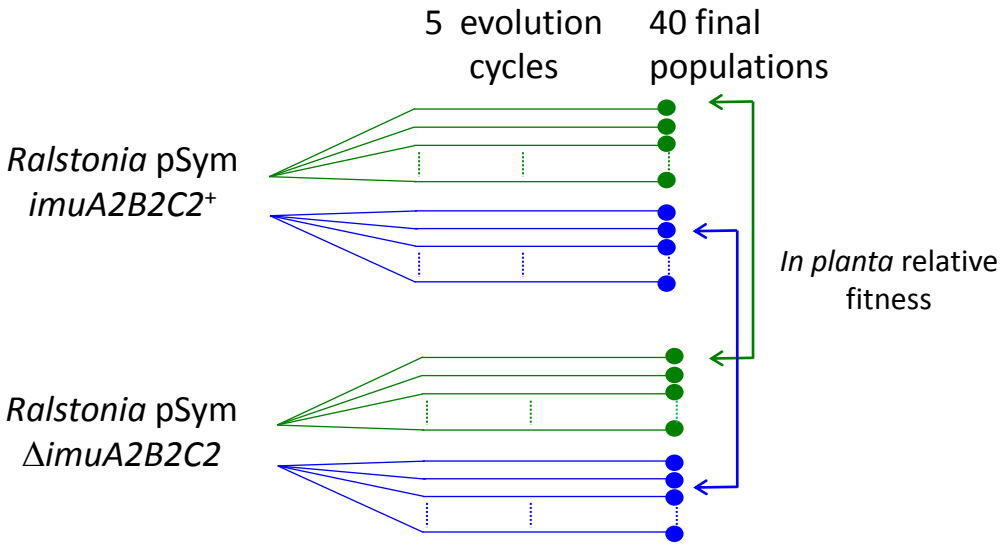


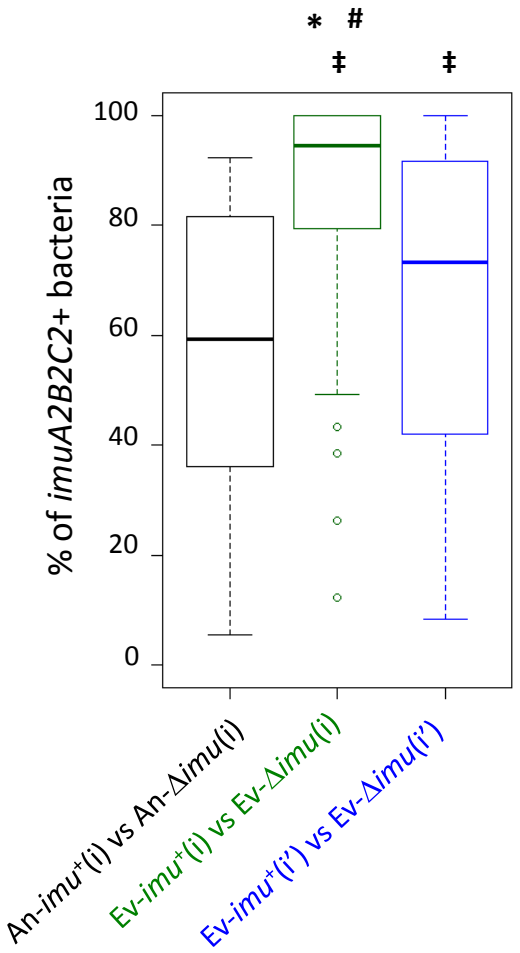
Two selection regimes:



Remigi *et al.,* PLoS Biol. 2014

How hypermutability impact on symbiotic evolution?



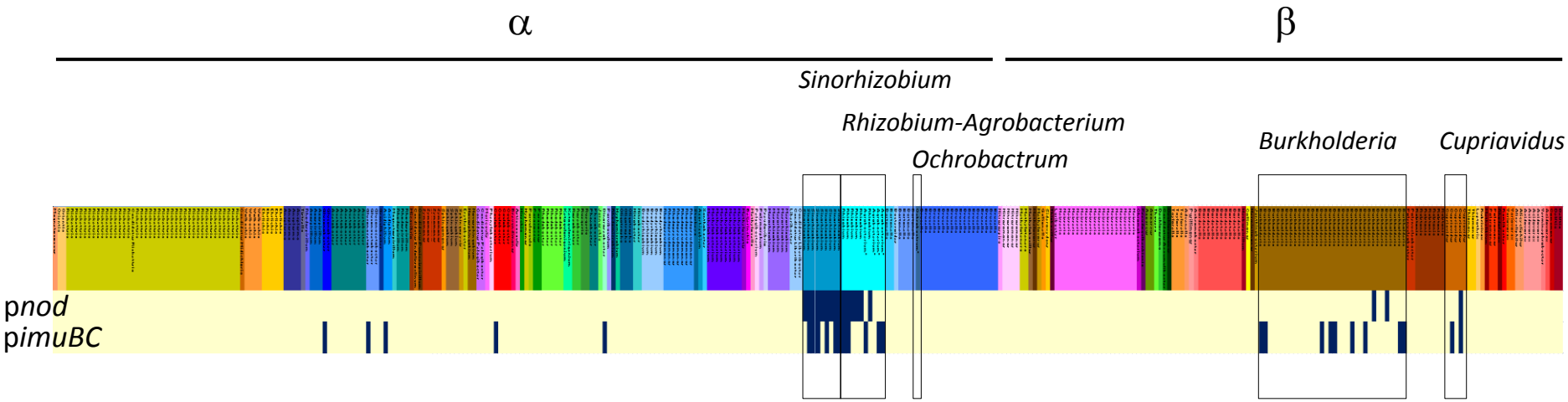


***imuA2B2C2*-dependent mutagenesis accelerates the symbiotic evolution of *Ralstonia* under *Mimosa* selection pressure**

Remigi *et al.,* PLoS Biol. 2014

Phylogenetic distribution of the plasmid *imuBC* cassettes

Among the 349 available genomes (109 genera) of α and β-proteobacteria, more than half possess a chromosomal *imuBC* cassette but only 28 have a plasmid cassette.

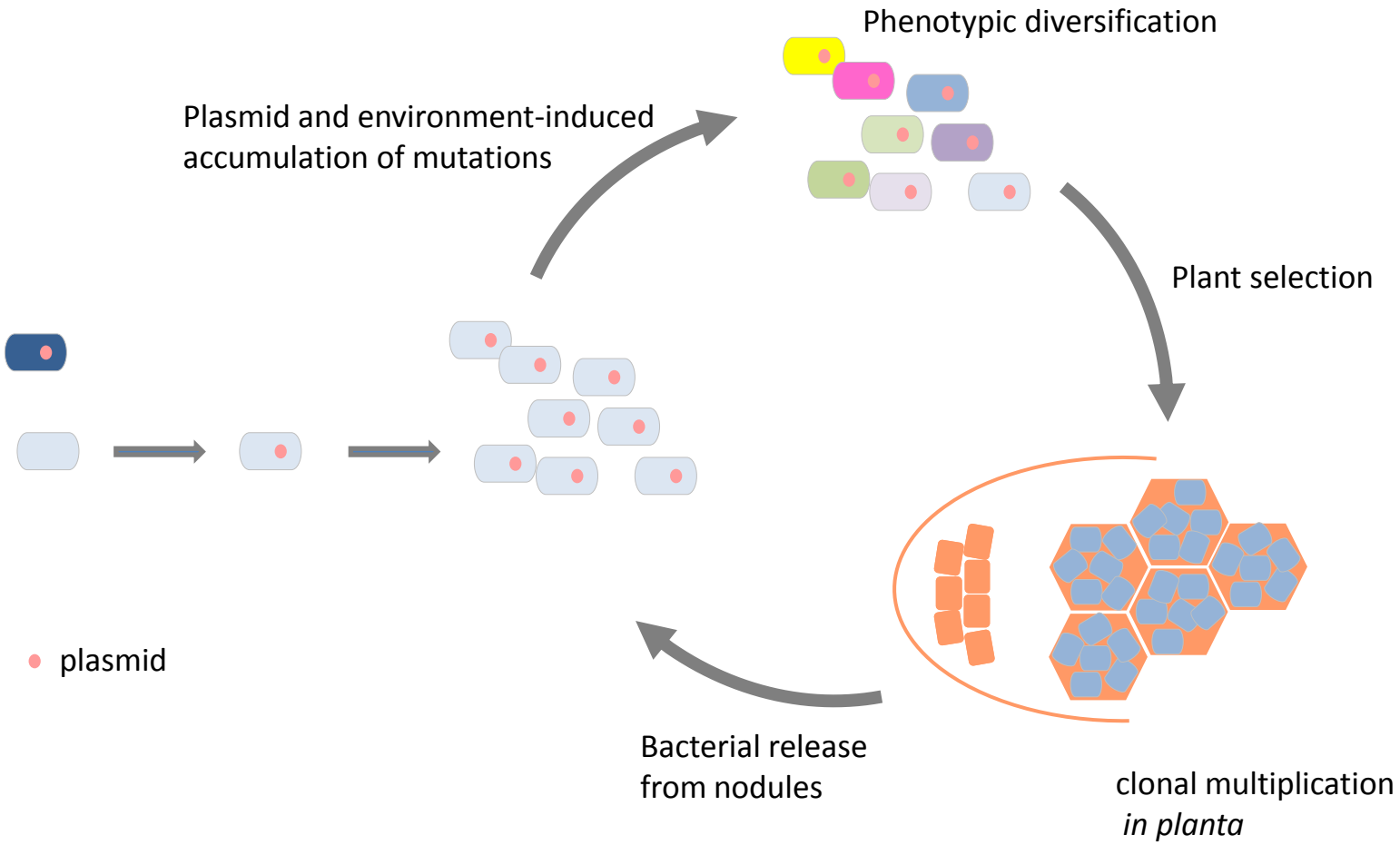


- 82% of all plasmid cassettes fall in the six genera that contain pSym-rhizobia
- 45% of the pSym possess a cassette

Plasmid *imuBC* cassettes may have favored dissemination of symbiotic genes within these taxa

Remigi *et al.,* PLoS Biol. 2014

Model for evolution of rhizobia



Remigi *et al.,* PLoS Biol. 2014

Conclusions

- We confirmed the remodelling of the recipient genome to express the symbiotic potential and adapt to legume symbiosis

- In some cases this process could have been accelerated by the co-transfer of mutagenic genes with symbiotic genes

- In our experiment, error-prone DNA polymerases encoded by the *C. taiwanensis* symbiotic plasmid trigger hypermutagenesis of the recipient genome, before bacteria enter the root

- This burst in genetic diversity has accelerated symbiotic evolution of *Ralstonia*

- ImuABC mutagenic cassettes are overrepresented in rhizobial lineages, supporting their role in rhizobium evolution

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