Origins of New Zealand's Rhizobia

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Centre for Biodiversity and Biosecurity Seminar
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Outline

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What are rhizobia?

Figure: *Acacia* root nodule

- Bacteria
- Specific symbiosis with legumes
- Form N_2-fixing root nodules

Native legumes

Figure: *Sophora chathamica*

Figure: *Clianthus puniceus*

Native legumes

Figure: *Carmichaelia australis*

Figure: *Montigena novae-zelandiae* © Peter Heenan

Introduced legumes

Figure: *Ulex europaeus* (Gorse)

Figure: *Cytisus scoparius* (Broom)

Introduced legumes

Figure: *Acacia longifolia*

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Legume arrival in NZ

Dates inferred by fossil pollen and phylogenetic trees.

- Native legumes arrived $\approx$ 5 million years ago
  - Presumably have co-evolved with native rhizobia.
- Exotic legumes arrived $<$ 200 years ago
  - With what rhizobia have they nodulated?

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Nodulation of introduced legumes

Possibilities for nodulation of introduced legumes

1. Nodulated by same rhizobia as native legumes.
2. Nodulated by introduced specific rhizobia.
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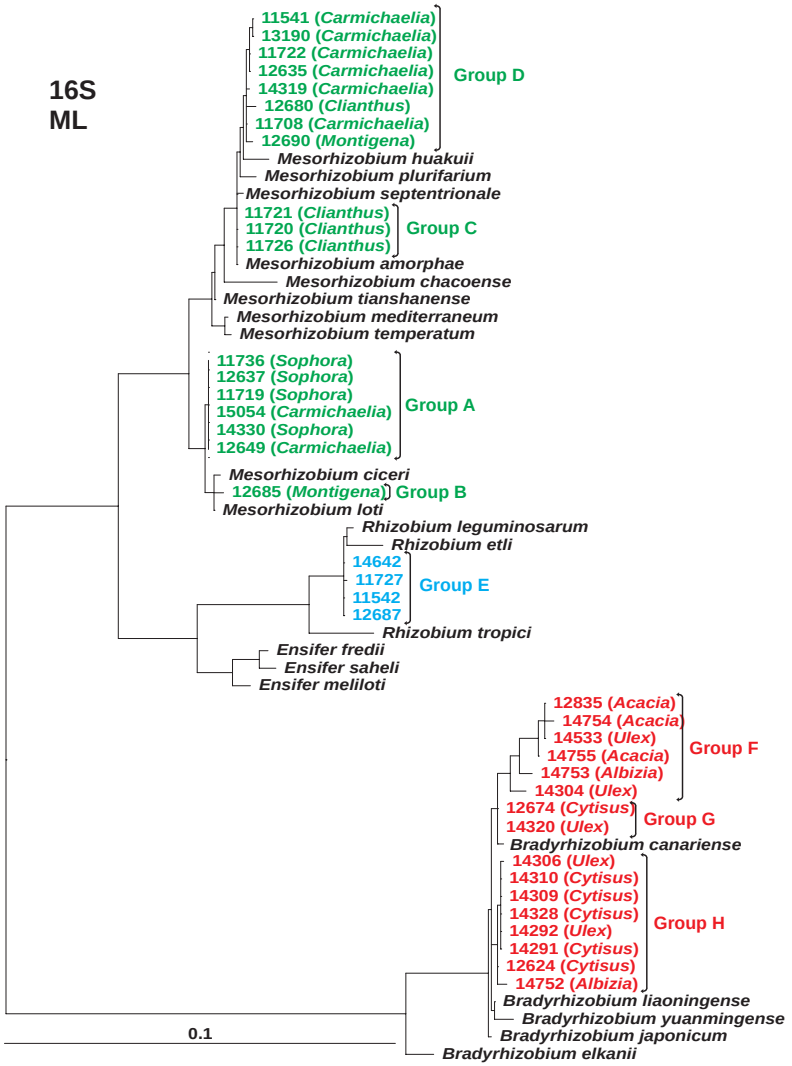
- Isolated 40 strains from root nodules.
- Sequenced 4 genes: 16S, *recA*, *atpD*, *glnII*.
- Built phylogenetic trees using NJ, ML, Bayesian, ProtTest.

Determined the identity of rhizobia



- High diversity.
- No relationship between rhizobial group and host.
- Native legumes $\longrightarrow$ *Mesorhizobium*.
- Introduced legumes $\longrightarrow$ *Bradyrhizobium*.
- Native and introduced legumes do not share rhizobia.

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- High diversity may indicate cosmopolitan bacteria with a long presence in NZ.
- But: The *Mesorhizobium–Bradyrhizobium* split was 500 mya, well before the evolution of legume symbiosis (< 80 mya).
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Nod factor

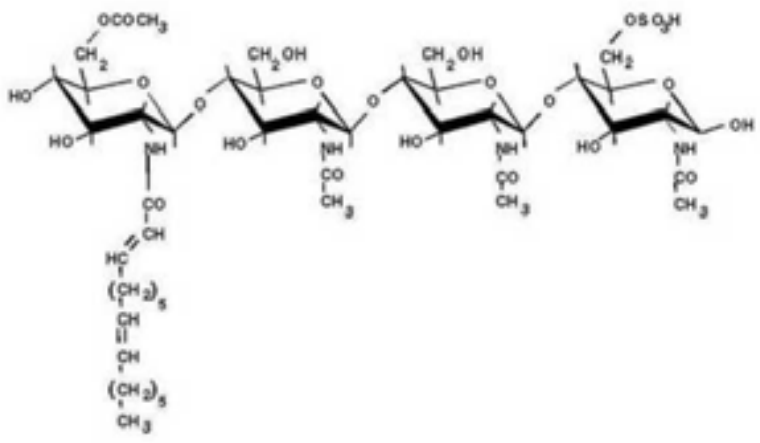

Figure: Nod factor (lipo-chito-oligosaccharide)

- *nod* genes form the nod factor.
- Critical molecule in nodule formation.
- Found on transmissible genetic elements.

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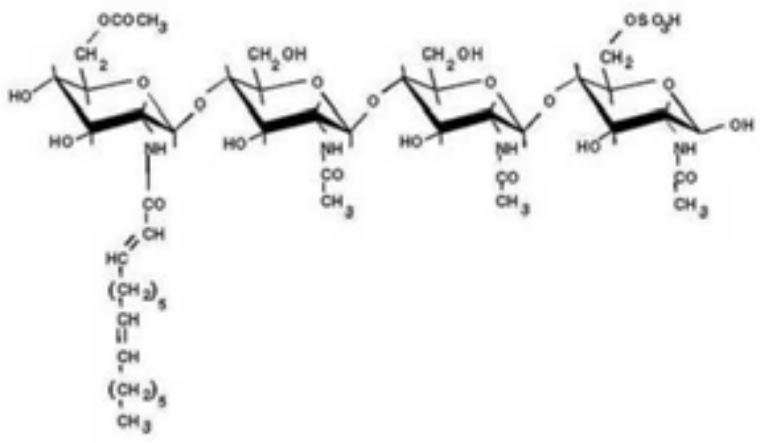

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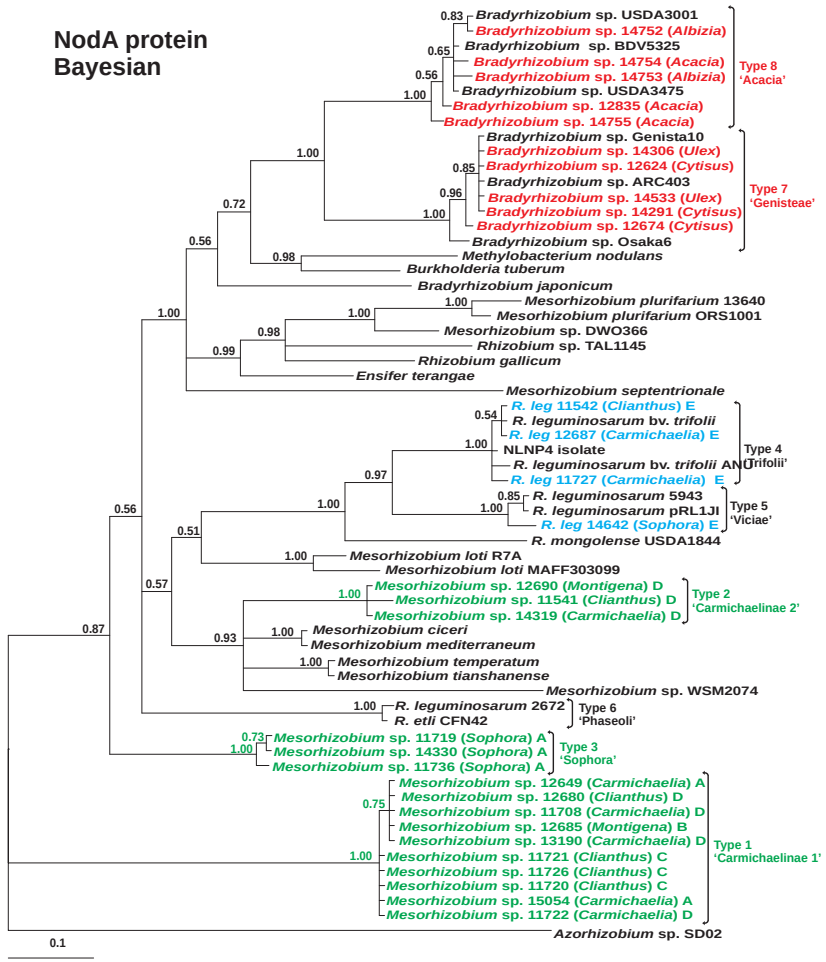
nodA gene



- Little correlation with house keeping genes.
  - Host specificity.
  - Horizontal gene transfer.
- Novel *nodA* genes in natives.
  - Genetic drift (wobble base).
  - Selection (during diversification).
- *nodA* genes of introduced are similar to overseas sequences.

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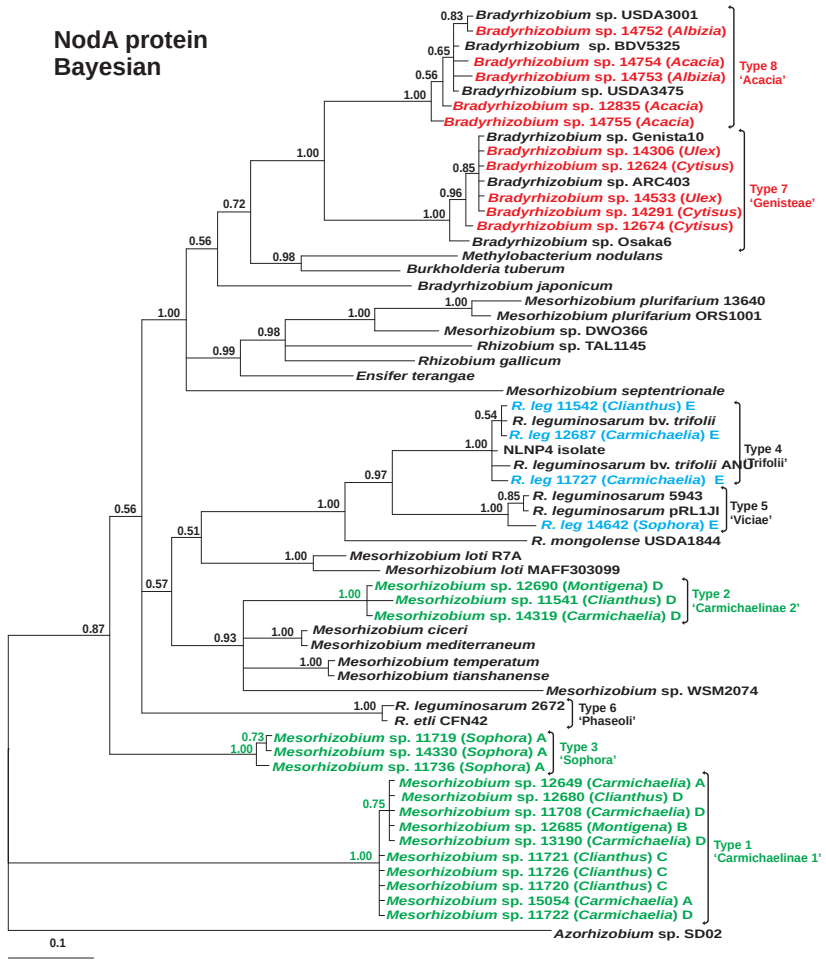

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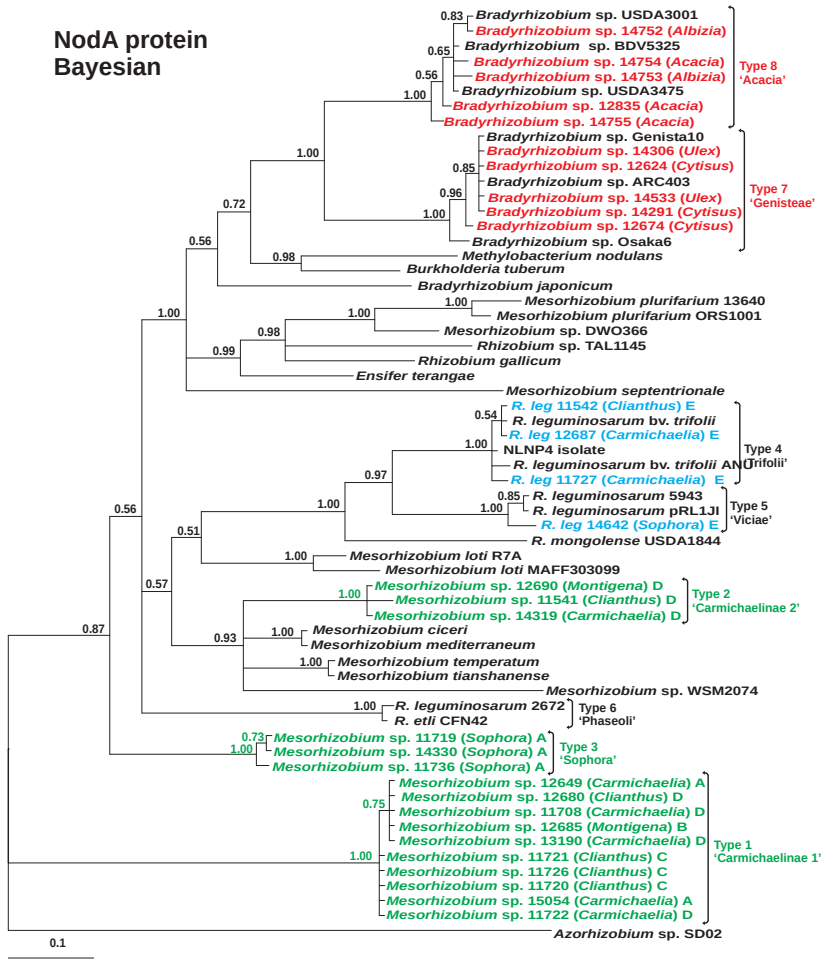

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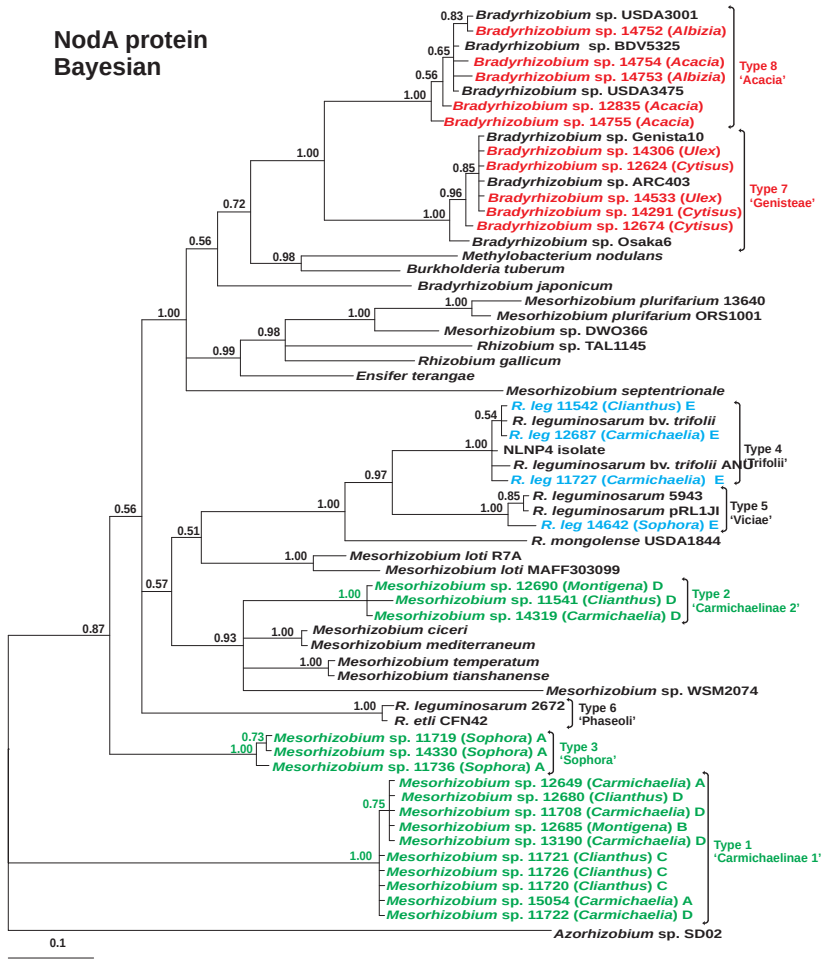

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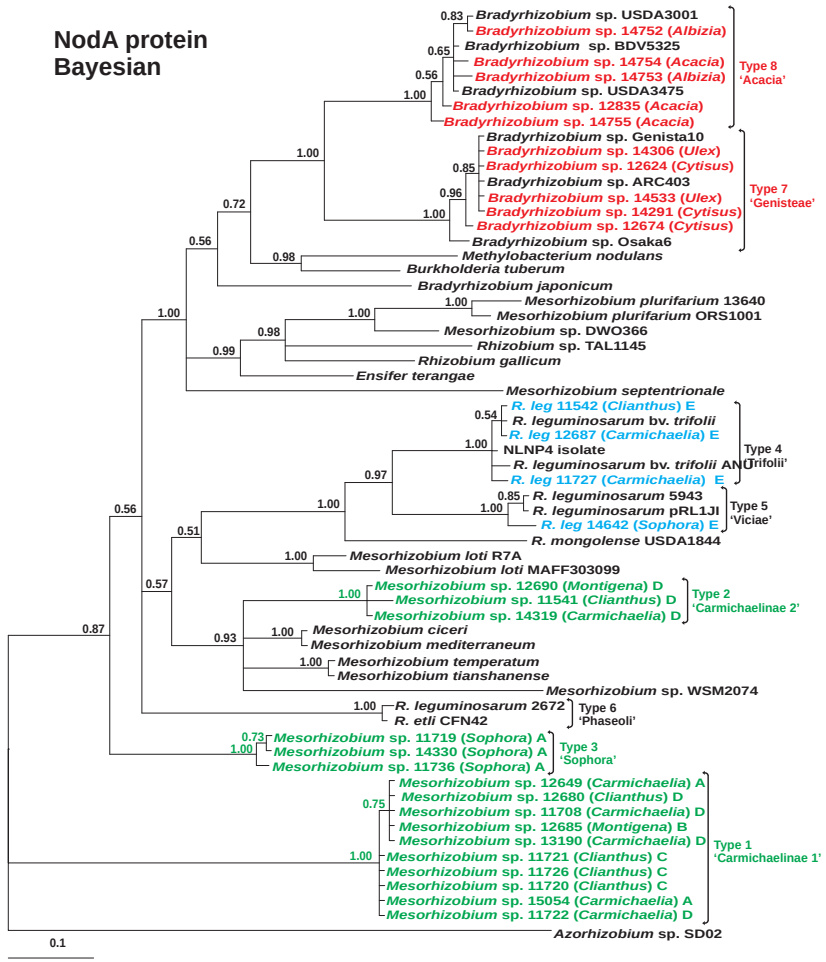

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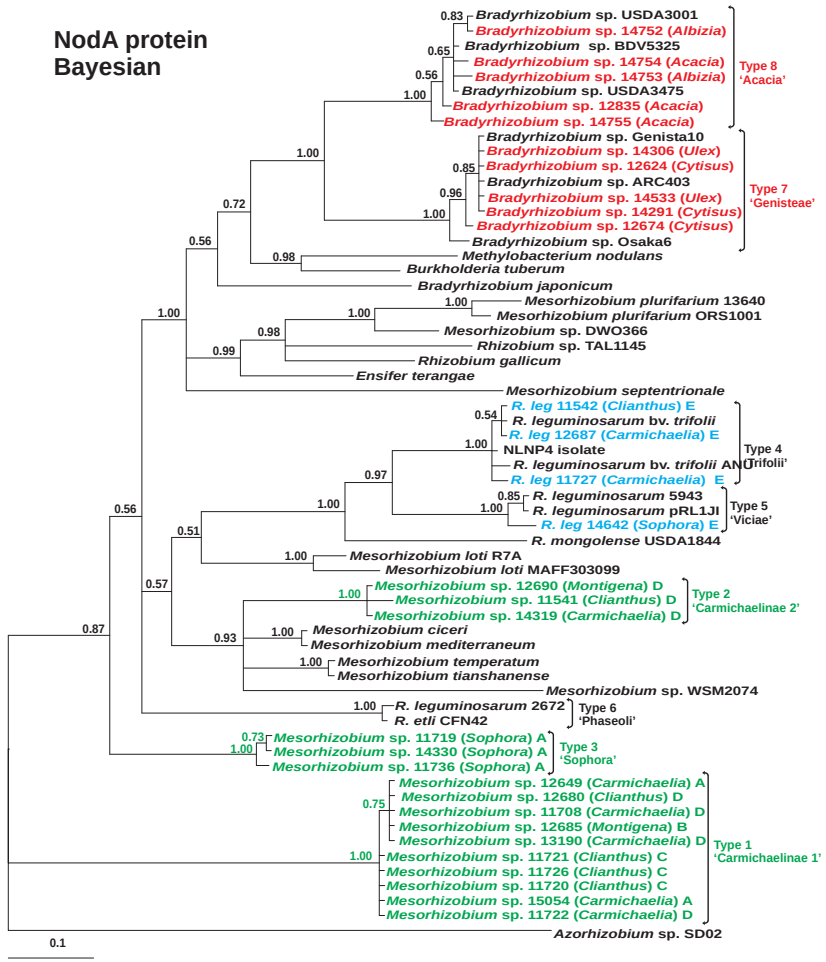

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Host-range experiments

Figure: *Sophora* growing in vermiculite

- Tested the ability of *Mesorhizobium* and *Bradyrhizobium* spp. to nodulate native and introduced species.

Methods

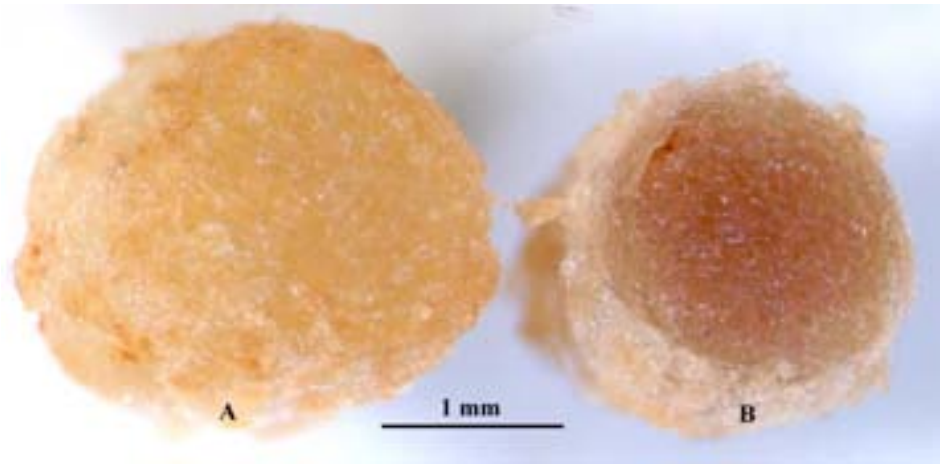

Figure: Ineffective and Effective *Clianthus* nodules

- Standard suspension of rhizobia.
- Sterile vermiculite, and seeds.
- 10 weeks growth.
- Nitrogenase assay, then uprooted and examined nodules.

Results

- Rhizobia isolated from native legumes (*Mesorhizobium* spp.) could generally effectively nodulate other native legumes (nod$^+$ fix$^+$).
  - Some strain variation
  - No clear link to *nodA* or genotype
- Rhizobia isolated from introduced legumes (*Bradyrhizobium* spp.) could generally effectively nodulate other introduced legumes (nod$^+$ fix$^+$).
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- *Rhizobium leguminosarum* formed ineffective nodules on native legumes
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Which other legumes can native rhizobia nodulate?

- Most likely candidates are those that nodulate with *Mesorhizobium* in other countries.
- Tested *Astragalus membranaceus* (milk vetch), *Lotus tetragonolobus* (asparagus pea), *Cicer arietinum* (chick pea), *Styphnolobium japonicum* (Japanese pagoda tree)
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Conclusion

Rhizobia travelled to NZ in association with their legume hosts from the centre of legume evolution in the northern hemisphere, to retain specificity.

Summary

- Native legumes are nodulated by *Mesorhizobium* spp. that are diverse, have novel *nod* genes, and nodulate only related species.
  - **Rhizobia origin:** Arrived 5+ mya, with legume ancestors.
- Introduced legumes are nodulated by *Bradyrhizobium* spp. that are diverse, and have *nod* genes similar to European (Gorse/Broom) and Australian (*Acacia*) species.
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Acknowledgements

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  - Dr. Susan Turner (Auckland University)
  - Prof. Warwick Silvester (Waikato University)
- Royal Society of New Zealand (Marsden Fund)
- Landcare Research

References I

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Unexpectedly diverse *Mesorhizobium* strains and *Rhizobium leguminosarum* nodulate native legume genera of New Zealand, while introduced legume weeds are nodulated by *Bradyrhizobium* species.
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