

# Origins of New Zealand's Rhizobia

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Landcare Research

Centre for Biodiversity and Biosecurity Seminar  
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# Outline

- 1 Introduction
  - Rhizobia and legumes
  - The Question: Origin of the rhizobia
- 2 Results and Discussion
  - Phylogenetic analyses
  - Nodulation gene analysis
  - Host-range testing



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# What are rhizobia?



Figure: *Acacia* root nodule

- Bacteria
- **Specific** symbiosis with legumes
- Form N<sub>2</sub>-fixing root nodules



# Native legumes



Figure: *Sophora chathamica*



Figure: *Clialthus puniceus*



# Native legumes



Figure: *Carmichaelia australis*



Figure: *Montigena novae-zelandiae* © Peter Heenan



# Introduced legumes



Figure: *Ulex europaeus* (Gorse)



Figure: *Cytisus scoparius*  
(Broom) © Jon J. Sullivan



# Introduced legumes



Figure: *Acacia longifolia* © Brenda Foran



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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

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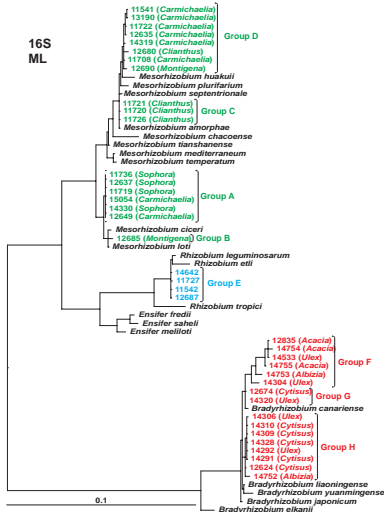


# Methods

- 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 → *Mesorhizobium*.
- Introduced legumes → *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

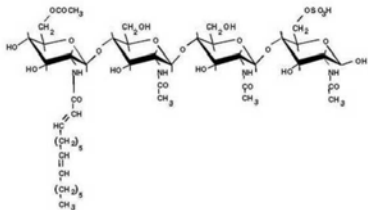


Figure: Nod factor  
(lipo-chito-oligosaccharide)

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



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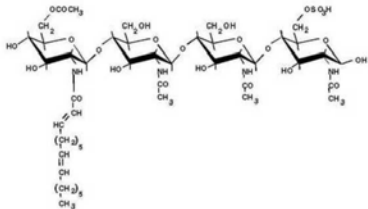


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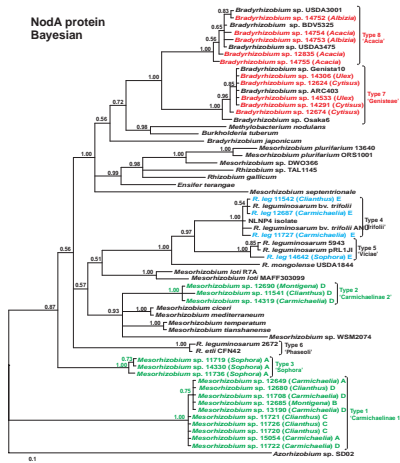
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# nodA gene

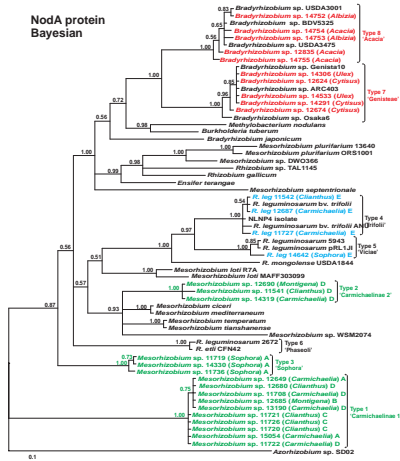


- Little correlation with house keeping genes.
  - Host specificity.
  - Horizontal gene transfer.
- Novel *nodA* genes in natives.
  - Genetic drift (wobble base).
  - Selection (during diversification).
- *nodA* genes of introduced are similar to overseas sequences.





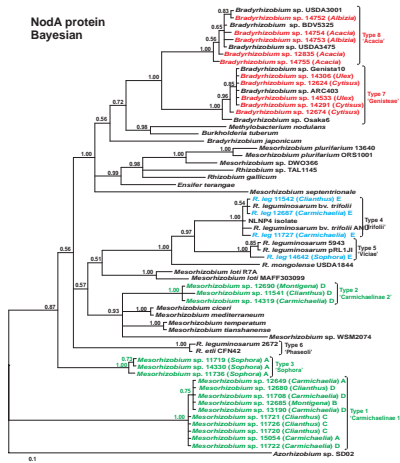
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# Host-range experiments

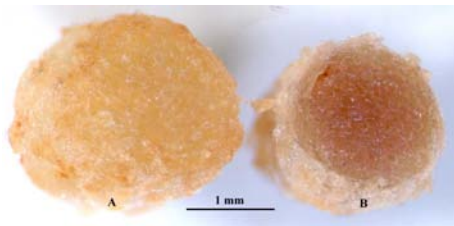


Figure: *Sophora* growing in vermiculite

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



# Methods



**Figure:** Ineffective and Effective *Clonanthus* 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<sup>+</sup> fix<sup>+</sup>).
  - 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<sup>+</sup> fix<sup>+</sup>).
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- *Rhizobium leguminosarum* formed ineffective nodules on native legumes
  - Parasitic.



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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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R. M. Greenwood (DSIR) 1960's and 70's.

- NZ rhizobia only effectively nodulate related legumes (Galegeae, Hedysareae, and Carmichaelinae tribes)
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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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- Future work:
  - *nodA* genes of rhizobia nodulating NZ legume relatives.
  - Structure of the nod factor, receptor, and other *nod* genes.



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# Acknowledgements

- My Phd Supervisors:
  - Dr. John Young (Landcare Research)
  - Dr. Susan Turner (Auckland University)
  - Prof. Warwick Silvester (Waikato University)
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- Landcare Research



# References I



Weir, B. S., Turner, S. J., Silvester, W. B., Park, D.-C., and Young, J. M.

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

*Applied and Environmental Microbiology*, 70(10): 5980–5987, 2004.

