

Rhizobial diversity in New Zealand legumes

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Abstract

Utilising inferred phylogenies from gene sequence data it is shown that in New Zealand different genera of rhizobial populations nodulate native legumes, and exotic invasive weeds. The diversity of strains discovered contrasts with previous studies implying host legume specificity. The host range of *Rhizobium leguminosarum* is extended by the observation that it nodulates native New Zealand woody legumes.



Montigena novae-zelandiae



Clianthus puniceus



Carmichaelia australis



Root nodule from *Acacia longifolia*



Acacia longifolia



Ulex europaeus (Gorse)



Cytisus scoparius (Broom)

Introduction

New Zealand has four genera of native legumes: *Sophora*, *Carmichaelia*, *Clianthus* and *Montigena*. Several exotic legumes (gorse, broom, *Acacia*) have reached "super weed" status covering millions of hectares, and costing tens of millions of dollars per year to control. Their success is due to, in part, their rhizobial symbioses.

New Zealand, as an island, has been separated from the regions of major legume evolution for about 80 million years, yet exotic legumes were introduced less than 200 years ago by early European settlers.

Objectives

The purpose of this study was to identify the rhizobia nodulating both native legumes and introduced weeds. Presumably the rhizobia associated with the native legumes have co-evolved over millions of years. But what species of rhizobia are the recently introduced legumes nodulating with?

There are three possibilities:

1. They have adapted to use native rhizobial populations
2. Effective rhizobia were co-introduced along with the exotic legumes
3. Effective rhizobial cosmopolitan populations are present in New Zealand soils.

The approach used in this study was to sequence four housekeeping genes, and examining their inferred phylogeny. Multiple genes were used to establish species level placement and to identify gene transfer events that may confuse phylogenetic inference.

Methods

- 36 bacterial isolates were obtained from the root nodules of 9 native and 5 introduced weed legume species from throughout the country.
- 5 type strains of rhizobia were selected to complement GenBank gene sequence data
- Four genes (16S rDNA, *atpD*, *glnII*, *recA*) PCR amplified and sequenced for 36 NZ strains and 5 type strains
- Phylogenetic trees were built for each gene using Neighbour-Joining and Maximum Likelihood methods, to assess the diversity and phylogenetic grouping of each strain, compared to type strains for selected rhizobial species.

Results

The phylogenetic trees produced were mostly congruent (only 16S rDNA shown here).

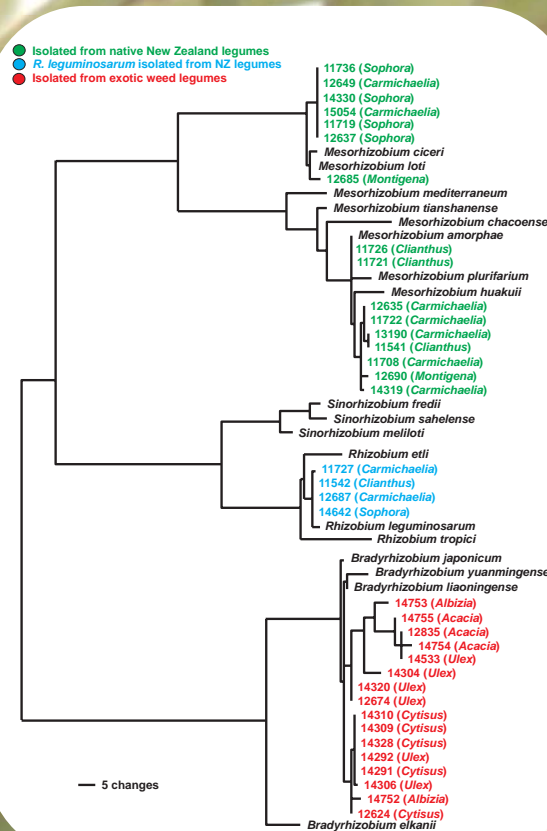
It is clear that native legumes and exotic legumes are nodulated by totally different rhizobial populations: Native legumes are nodulated by diverse *Mesorhizobium* populations and by *Rhizobium leguminosarum*, whilst introduced legumes are nodulated by diverse *Bradyrhizobium* spp.

No geographical distribution correlation was evident (data not shown).

The question of origin is now partially solved, the exotic legumes in this study must be using introduced or cosmopolitan rhizobial populations.

Additionally four native legumes were found to nodulate with isolates of *Rhizobium leguminosarum* – this represents an extension to the host range of this species. These woody New Zealand legumes are very different to the currently accepted host range of the three biovars of *R. leguminosarum*.

The widespread distribution of strains from individual native legume genera across *Mesorhizobium* contrast with previous reports that imply the specificity of bacterial species to limited numbers of legume genera.



Maximum Likelihood phylogenetic tree showing the relationship of rhizobia isolated from legumes present in New Zealand, compared to type strains. Host legume genus is indicated in the parentheses.