Identification and Specificity of Rhizobia in New Zealand

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NZ Native Legumes

- Kowhai (*Sophora* spp.)
- Kaka beak (*Clianthus* sp.)
- NZ Broom (*Carmichaelia* spp.)
- Scree Pea (*Montigena* sp.)
Questions - Natives

• What rhizobia are present in NZ?
  – NZ split 80 mya, became isolated
  – Co-evolution between native legumes and rhizobia since that time

• How specific are the relationships that have formed since that time?
  – Does each legume species/genus have its own rhizobia or are the relationships more general
Woody Legume Weeds in NZ

1-2 million hectares, tens of millions $ pa.

- Gorse (*Ulex europaeus*)
- Broom (*Cytisus scoparius*)
- Wattles (*Acacia* spp.)

Gorse (*Ulex europaeus*)

Broom in Porters Pass, South Island

Acacia longifolia
Questions - Exotics

- Weed legumes were introduced
  - Present for less than 200 years
- How are the weeds able to nodulate?
- Three possibilities:
  - They have adapted to use native rhizobial populations
  - Effective rhizobia were co-introduced with the exotic legumes
  - Effective cosmopolitan rhizobial populations are present in New Zealand
Research Strategy

• **Identification**
  – Multiple gene sequencing, phylogenetics
  – Biolog
  – Fatty acids

• **Specificity**
  – Nodulation gene
  – Host-range: Inoculate native and exotic legumes with rhizobial strains, acetylene reduction, nodule counting.
Phylogenetics

- Isolated 40 strains from root nodules

- Sequenced 5 genes
  - 16S, recA, atpD, glnII, nodA

- Built phylogenetic trees
  - NJ, ML, Bayesian
  - ProtTest, Modeltest
16S Phylogeny of NZ Rhizobia

Native legumes - *Mesorhizobium*

*Rhizobium leguminosarum*

Exotic legumes - *Bradyrhizobium*
Nodulation genes

- Nod factor is a bacterial signal molecule that initiates nodule formation

- Nod factor “core” created by three genes:
  - $nodA$, $nodB$, $nodC$
  - Some degree of relation to host range

- Sequenced $nodA$:
  - reveal evolutionary history of the gene, and
  - relation to specificity in NZ legumes
nodA phylogeny

• Three novel nodA types in NZ Mesorhizobium
  – No correlation with house-keeping genes
    • Carried on transposable symbiosis island
  – No correlation with host legume
• R. leguminosarum strains have typical nodA
  – Haven't picked up Mesorhizobium symbiosis island
• Bradyrhizobium nodA similar to others found
  – Possibly host specific
Research Strategy

• **Identification**
  – Multiple gene sequencing, phylogenetics
  – Biolog (not shown)
  – Fatty acids (not shown)

• **Specificity**
  – Nodulation gene
  – Host-range: Inoculate plants with strains, acetylene reduction, nodule counting.
Research Objectives for Specificity

• Investigate symbiotic promiscuity of *Mesorhizobium* strains nodulating native NZ legumes.

• Investigate symbiotic promiscuity of *Bradyrhizobium* strains nodulating exotic weed legumes.

• Investigate interactions between *R. leguminosarum* and native NZ legumes.

• Verify the previously established division of *Mesorhizobium* nodulating natives, and *Bradyrhizobium* nodulation exotics.
Mesorhizobium – Native Legumes

- 10 Mesorhizobium strains
- 5 Native legumes
  - Two Sophora
  - Two Carmichaelia
  - Clianthus
- Five replicates, uninoculated controls, Full cross inoculation, vermiculite, acetylene reduction

Cross section through Clianthus nodules
<table>
<thead>
<tr>
<th>Mesorhizobium strain</th>
<th>Genomic group</th>
<th>nodA type</th>
<th>(\text{nod/fix response on inoculated native legume})</th>
</tr>
</thead>
<tbody>
<tr>
<td>14330 ((\text{Sophora}))</td>
<td>A</td>
<td>3</td>
<td>(\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^-) (\text{nod}^+) (\text{fix}^-) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>11719 ((\text{Sophora}))</td>
<td>A</td>
<td>3</td>
<td>(\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^-) (\text{nod}^+) (\text{fix}^-) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>15054 ((\text{Carmichaelia}))</td>
<td>A</td>
<td>1</td>
<td>(\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>12685 ((\text{Montigena}))</td>
<td>B</td>
<td>1</td>
<td>(\text{nod}^-) (\text{fix}^-) (\text{nod}^-) (\text{fix}^-) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>11721 ((\text{Clianthes}))</td>
<td>C</td>
<td>1</td>
<td>(\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>11726 ((\text{Clianthes}))</td>
<td>C</td>
<td>1</td>
<td>(\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>11541 ((\text{Clianthes}))</td>
<td>D</td>
<td>2</td>
<td>(\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>12680 ((\text{Clianthes}))</td>
<td>D</td>
<td>1</td>
<td>(\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>12690 ((\text{Montigena}))</td>
<td>D</td>
<td>2</td>
<td>(\text{nod}^-) (\text{fix}^-) (\text{nod}^-) (\text{fix}^-) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+)</td>
</tr>
<tr>
<td>13190 ((\text{Carmichaelia}))</td>
<td>D</td>
<td>1</td>
<td>(\text{nod}^-) (\text{fix}^-) (\text{nod}^-) (\text{fix}^-) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+) (\text{nod}^+) (\text{fix}^+)</td>
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</table>
Mesorhizobium – Native Legumes

- Generally broad nodulation
  - All nodulated *Clianthus* effectively
- Exceptions: *Sophora*
  - 3 strains unable to form nodules, 1 ineffective
  - 2 strains isolated from *Sophora* ineffective on *Carmichaelia*
- No clear correlation to genomic or *nodA* groups
- Just how broad is the symbiosis? …
Mesorhizobium – Exotic Legumes

- Broad nodulation in natives
  - Can they nodulate other legumes?
- Best possible targets:
  - Chick Pea (*Cicer*), Milk Vetch (*Astralagus*), *Lotus*, other *Sophora*

- Inoculated four exotic legumes with seven *Mesorhizobium* strains
# Mesorhizobium – Exotic Legumes

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<thead>
<tr>
<th>Mesorhizobium strain</th>
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<th>nodA type</th>
<th>nod/fix response on inoculated legume species</th>
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<tr>
<td>14330 (<em>Sophora</em>)</td>
<td>A</td>
<td>3</td>
<td>nod⁺ fix⁺</td>
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<tr>
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<td>A</td>
<td>1</td>
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<td>1</td>
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<td>C</td>
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<td>1</td>
<td>nod⁺ fix⁺</td>
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</table>
Mesorhizobium – Exotic Legumes

• Only Astragalus nodulated
  – No correlation to genomic or nodA type

• Wagstaff et al. (1999): Astragalus, Clianthus, Carmichaelia are closely related.
  – Lessertia, Sutherlandia, Swainsona, Colutea [Galegeae]
  – NZ Mesorhizobium nodulate the Galegeae tribe and related

• Interesting that some strains nodulated exotic Astragalus, but not some native legumes
Rhizobium leguminosarum

- Four isolates identified as *R. leguminosarum*
  - Thought to nodulate peas, bean, clover
  - Host range extended through GM
- Hypothesis is that they are contaminants
  - Clover innoc. Widely used
- Tested the four isolates, plus one each of the three biovars: *trifolii, phaseoli, viceae*
- Inoculated 3 native legumes, and three typical hosts (pea, bean, clover)
## Rhizobium leguminosarum

<table>
<thead>
<tr>
<th>Rhizobium leguminosarum strain</th>
<th>nodA type</th>
<th>nod/fix response on inoculated legume species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sophora microphylla</td>
</tr>
<tr>
<td>11542 (Clanthus)</td>
<td>4</td>
<td>nod⁻ fix⁻</td>
</tr>
<tr>
<td>11727 (Carmichaelia)</td>
<td>4</td>
<td>nod⁻ fix⁻</td>
</tr>
<tr>
<td>12687 (Carmichaelia)</td>
<td>4</td>
<td>nod⁻ fix⁻</td>
</tr>
<tr>
<td>14642 (Sophora)</td>
<td>5</td>
<td>nod⁺ fix⁻</td>
</tr>
<tr>
<td>2668 (trifoli)</td>
<td>4</td>
<td>nod⁻ fix⁻</td>
</tr>
<tr>
<td>2672 (phaseoli)</td>
<td>6</td>
<td>nod⁻ fix⁻</td>
</tr>
<tr>
<td>5943 (viceae)</td>
<td>5</td>
<td>nod⁻ fix⁻</td>
</tr>
</tbody>
</table>
Rhizobium leguminosarum

- *R. leguminosarum* strains nodulate NZ legumes but ineffectively
- *R. leguminosarum* from natives nodulated pea, bean and clover effectively
- Probably typical of most *R. leguminosarum* strains
  - Novel host range expansion

Nodulation in Pea
Bradyrhizobium - Exotic Legumes

• 4 *Bradyrhizobium* strains, isolated from *Acacia*, gorse, and broom

• 3 exotic legumes:
  – *Acacia*, gorse, and broom

• Full cross inoculation
<table>
<thead>
<tr>
<th><em>Bradyrhizobium</em> strain</th>
<th>Genomic group</th>
<th><em>nodA</em> type</th>
<th><strong>nod/fix response</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>12674 (<em>Ulex</em>)</td>
<td>I</td>
<td>7</td>
<td><em>nod</em>⁺ <em>fix</em>⁺</td>
</tr>
<tr>
<td>14291 (<em>Cytisus</em>)</td>
<td>J</td>
<td>7</td>
<td><em>nod</em>⁺ <em>fix</em>⁺</td>
</tr>
<tr>
<td>14533 (<em>Ulex</em>)</td>
<td>G</td>
<td>7</td>
<td><em>nod</em>⁺ <em>fix</em>⁺</td>
</tr>
<tr>
<td>14755 (<em>Acacia</em>)</td>
<td>G</td>
<td>8</td>
<td><em>nod</em>⁺ <em>fix</em>±</td>
</tr>
</tbody>
</table>

**Cytisus scoparius**  | *Ulex europaeus*  | *Acacia longifolia* |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>nod</em>⁺ <em>fix</em>⁺</td>
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<td><em>nod</em>⁺ <em>fix⁻</em></td>
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</table>
Bradyrhizobium - Exotic Legumes

• Broad nodulation
  – All strains nodulated all legumes

• Gorse and Broom isolates ineffective on Acacia
  – Correlation to nodA gene
Conclusions

• The native legumes are effectively nodulated by diverse *Mesorhizobium* populations that have a host range encompassing all of the native legumes (and other members of the Galegeae).

• The native legumes are also nodulated — albeit ineffectively — by some strains of *Rhizobium leguminosarum*.
Conclusions

• The exotic weed legumes: Broom, Gorse, *Acacia* are effectively nodulated by diverse *Bradyrhizobium* species, and can cross nodulate.

• Confirmed that *Bradyrhizobium* strains do not nodulate the native legumes, and *Mesorhizobium* do not nodulate exotic weed legumes.
Origins of Bradyrhizobia?

• Three possibilities of nodulating exotic weeds:
  – They have adapted to use native rhizobial populations
  – Effective rhizobia were co-introduced with the exotic legumes
  – Effective cosmopolitan rhizobial populations are present in New Zealand

• If introduced: would expect a different distribution in pristine soils than disturbed soils

• Investigated by baiting NZ soils with trap hosts…
Soil Investigations

- Soil samples were collected from:
  - *Carmichaelia* and *Sophora* rhizospheres
  - Broom and gorse rhizospheres
  - pristine soils (legumes not in sight)
- Soils into pots, then planted with native and introduced legumes
- Examined for nodulation after 16 weeks
- Rhizobia isolated and identified (sequencing)
Results

Bait plants

<table>
<thead>
<tr>
<th>Nodules/pot Category</th>
<th>Native</th>
<th>Intro</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td></td>
<td></td>
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<tr>
<td>= 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rhizosphere  Pristine  Rhizosphere
Summary

• Little or no nodulation occurs when native legumes are sown in pristine soils
  - consistent with earlier reports (Greenwood 1978; Sullivan & Ronson 1997); Mesorhizobia are present in soil but do not nodulate - perhaps because they lack the symbiosis island

• Nodulation of introduced legumes readily occurs when sown in pristine soils
  - consistent with a concept of cospolitain Bradyrhizobia nodulating introduced legumes
More Information

• Web: http://www.rhizobia.co.nz
• Email: info@rhizobia.co.nz
• Weir et. al. (2004) AEM 70(10): 5980-5987
• Endangered native *Clianthis* forms effective symbiosis with all *Mesorhizobium* spp.
• But… also forms ineffective nodules with *R. leguminosarum*, could compete.
• Need to think about “contaminating” national parks with foreign rhizobia