Landcare Research
Manaaki Whenua

"*Candidatus* Liberibacter ssp" and broom psyllids

Dagmar Goeke
Ecological Genetics group, Landcare Research

Biosecurity Bonanza

Christchurch, 20th May 2013

1) The “host”:
Common or Scotch broom - *Cytisus scoparius*

- Broom is native to Europe
- Spread worldwide as an ornamental
- Major weed in NZ, Australia, USA, India, Japan, Chile, Argentina
- Invades low value pasture, newly planted forestry, and natural habitats (grasslands, sub-alpine scrub, braided rivers).
- Net benefit to NZ if biocontrol successful $10m/yr*

* Jarvis, P. J. et al. 2006. Biol. Control 39, 135-146

2) The “venue”:
Biological Control of Broom in NZ

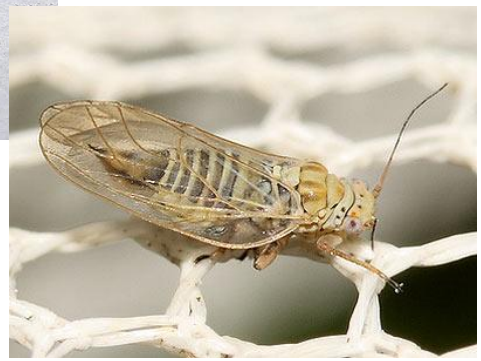
Existing exotic biocontrol agents:

- Twig miner, *Leucoptera spartifoliella* 1940s
- Broom seed beetle, *Bruchidius villosus*
- **Broom psyllid**, *Arytainilla spartiophila*
- Soft shoot moth, *Agonopteryx assimilella*
- Leaf beetle, *Gonioctena olivacea*
- Gall mite, *Aceria genistae*

Plots established at Hanmer and Waiouru to measure long-term impact using insecticide exclusion

3) The imported “vector”:
NZ broom psyllid - *Arytainilla spartiophila*

- Originates from UK
- First release in NZ in 1993, in Australia in 1994
- Agent impact: good in Southland, occasionally elsewhere



http://www.britishbugs.org.uk/homoptera/Psylloidea/Arytainilla_spartiophila.html

Risk Assessment in Weed Biocontrol

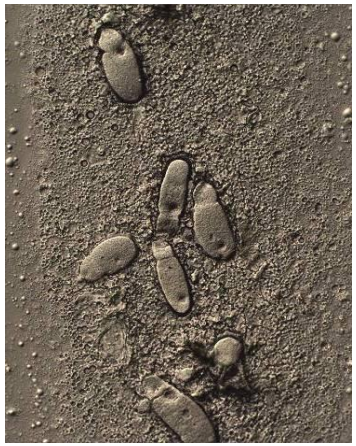
- **Host range testing** – with particular focus on NZ natives and valued exotics that are related to broom

- Purpose: **assess risk of non-target damage**

- **Broom psyllid was not tested for "*Ca.* Liberibacter spp",**
  - as it wasn't a known risk then and
  - there was no existing technique for identification

"*Candidatus* Liberibacter ssp"

- Non-culturable, bacterium-like prokaryotes
- DNA techniques needed for identification
- vectored by psyllids



"*Candidatus* Liberibacter ssp"

- Non-culturable, bacterium-like prokaryotes
- DNA techniques needed for identification
- vectored by psyllids

- Known "*Candidatus* Liberibacter ssp" :

  1-3) "*Ca.* L. asiaticus / africanus / americanus"
  cause *huánglóngbìng* (citrus greening disease)

  4) "*Ca.* L. solanacearum" (Lso) causes tomato/potato yellows disease and zebra chip disease

  5) "*Ca.* L. europeaus" *(*Leu) in pear – non-pathogenic

- 6) *Liberibacter crescens* in papaya – non-pathogenic relative to Las

4) The uninvited "guest": *"Ca.* L. europaeus" (Leu)

- Plant&Food observe Lso disease symptoms in broom in early in 2012[1)]

- DNA sequence analysis (16S rRNA and 16S-23S) of broom and broom psyllids:
  "*Ca.* L. europaeus " infestation of both

- first appearance in Italy in 2010 in pear and pear psyllids (behaves as an endophyte) [2)]

1) Thompson *et al.* 2013. First report of '*Candidatus* Liberibacter europaeus' associated with psyllid infested Scotch broom. New Disease Reports 27, 6.

2) Raddadi N *et al* .2011. '*Candidatus* Liberibacter europaeus' sp. nov. that is associated with and transmitted by the psyllid Cacopsylla pyri apparently behaves as an endophyte rather than a pathogen. Environmental Microbiology 13, 414-426.

???

- **Leu imported** with broom psyllid **or** is it **endemic**?

- **"Host"-specificity**
  - of Leu – pear, broom, and anything else?
  - of psyllids – probing on non-hosts?

- **"Vector"-specificity of Leu** – other than pear and broom psyllids or creeping plants?

- **Impact of Leu**
  - on NZ broom – reason for successful biocontrol?
  - on psyllids – plain vector function?
  - on NZ native flora – pathogenic or asymptomatic

Potential hosts and vectors of Leu in NZ

Hosts:

- NZ native Fabaceae e.g. *Carmichaelia*, *Clianthus*, *Sophora*
- Exotics – ornamental species (e.g. *Cytisus* cultivars)

Vectors:

- Endemic psyllids on *Sophora* and *Carmichaelia*
- Other psyllids such as Australian acacia psyllids

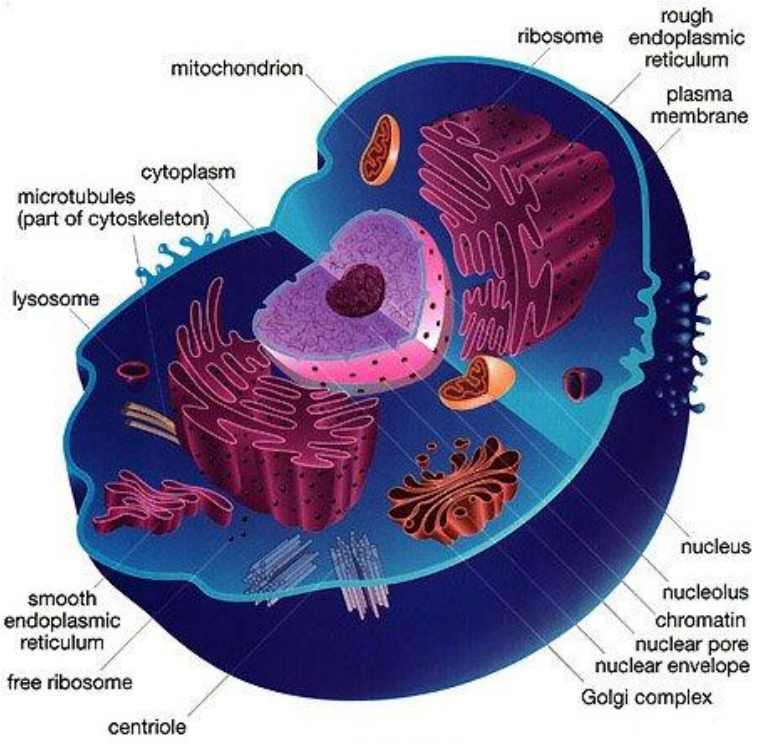
Approaching genetic diversity

- **DNA sequence analysis – psyllid species identification**

- Real-time PCR – Leu infestation testing of host and vector

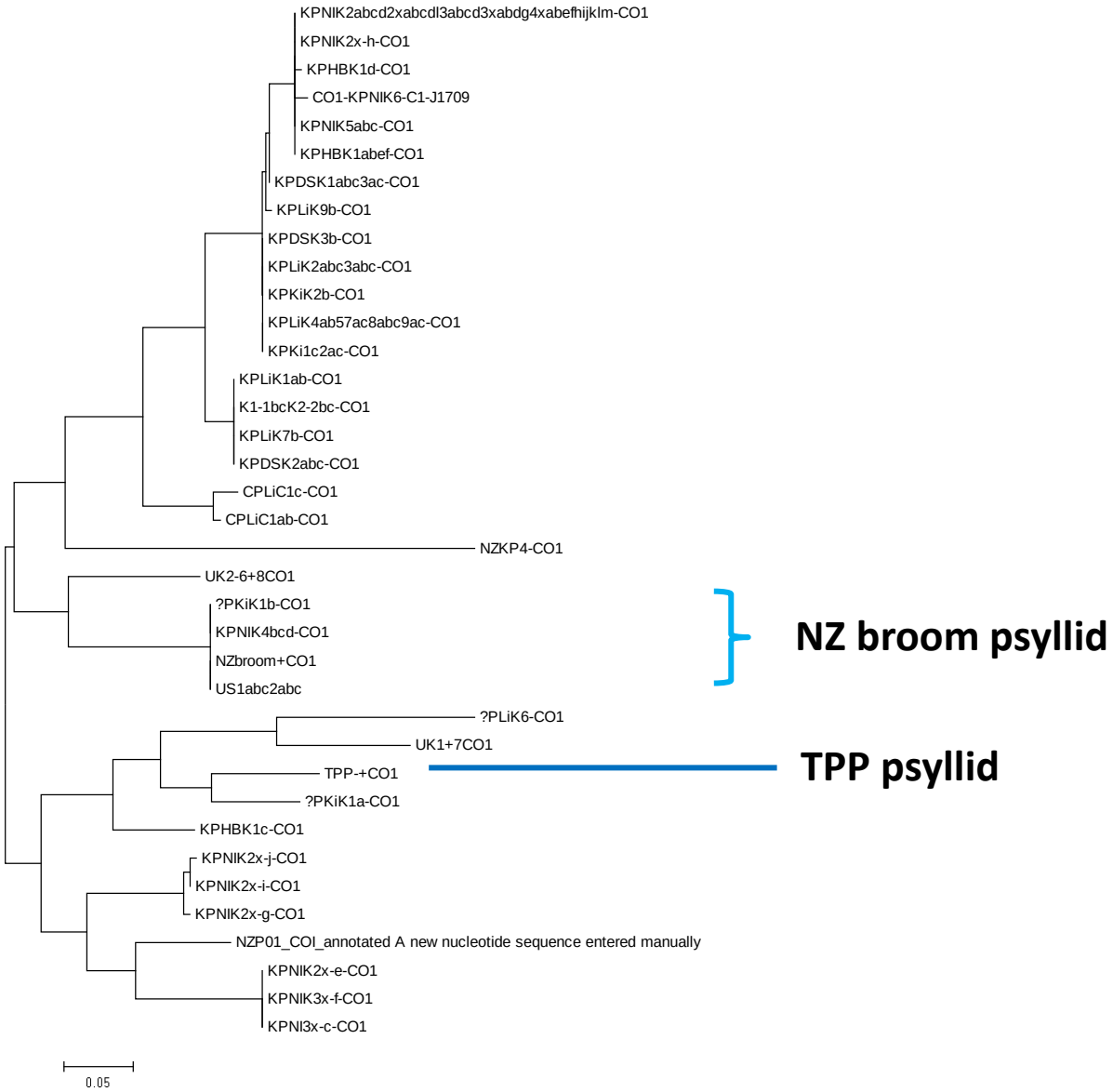
Psyllid DNA sequence data

CO1 (mitochondrial DNA region)

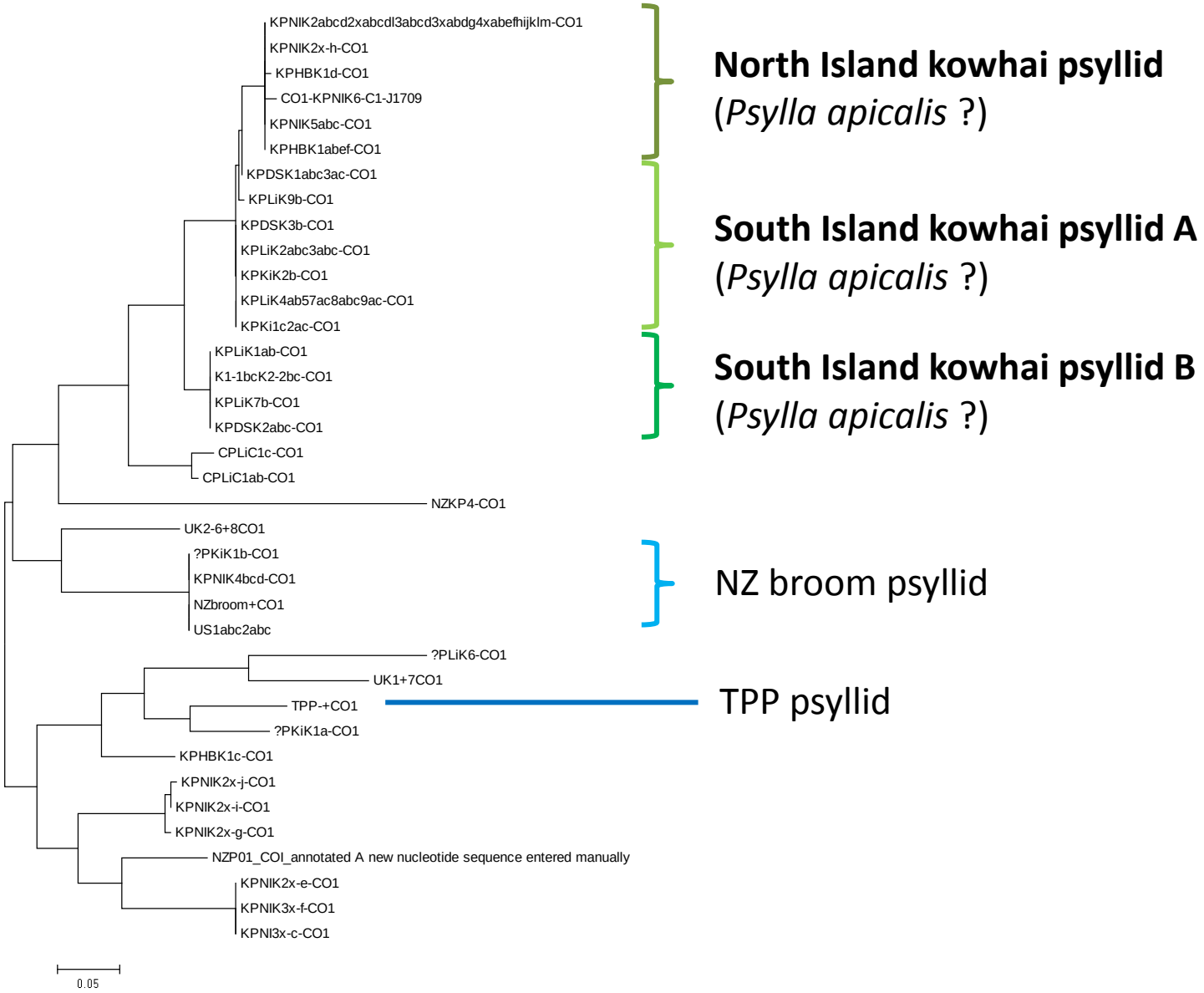


http://journalofcosmology.com/Extinction101.html

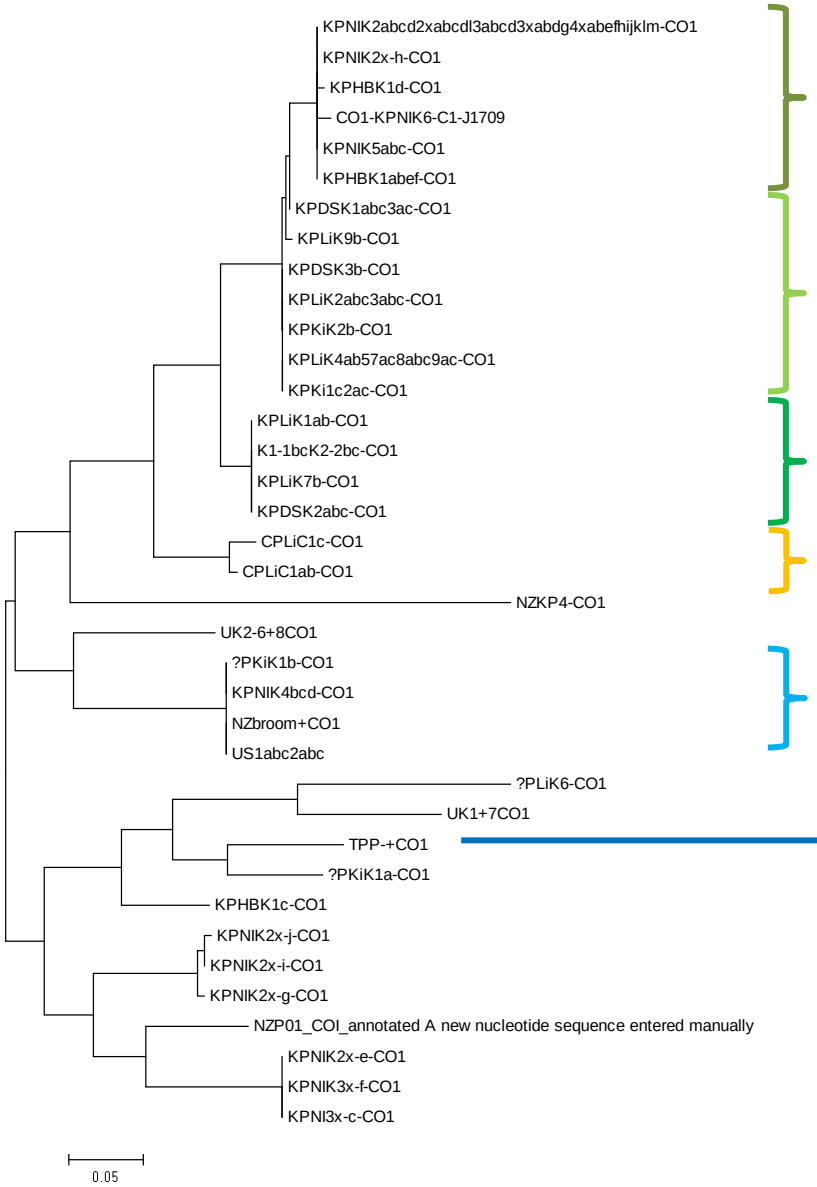
Sequence data analysis



Sequence data analysis



Sequence data analysis



North Island kowhai psyllid (*Psylla apicalis* ?)

South Island kowhai psyllid A (*Psylla apicalis* ?)

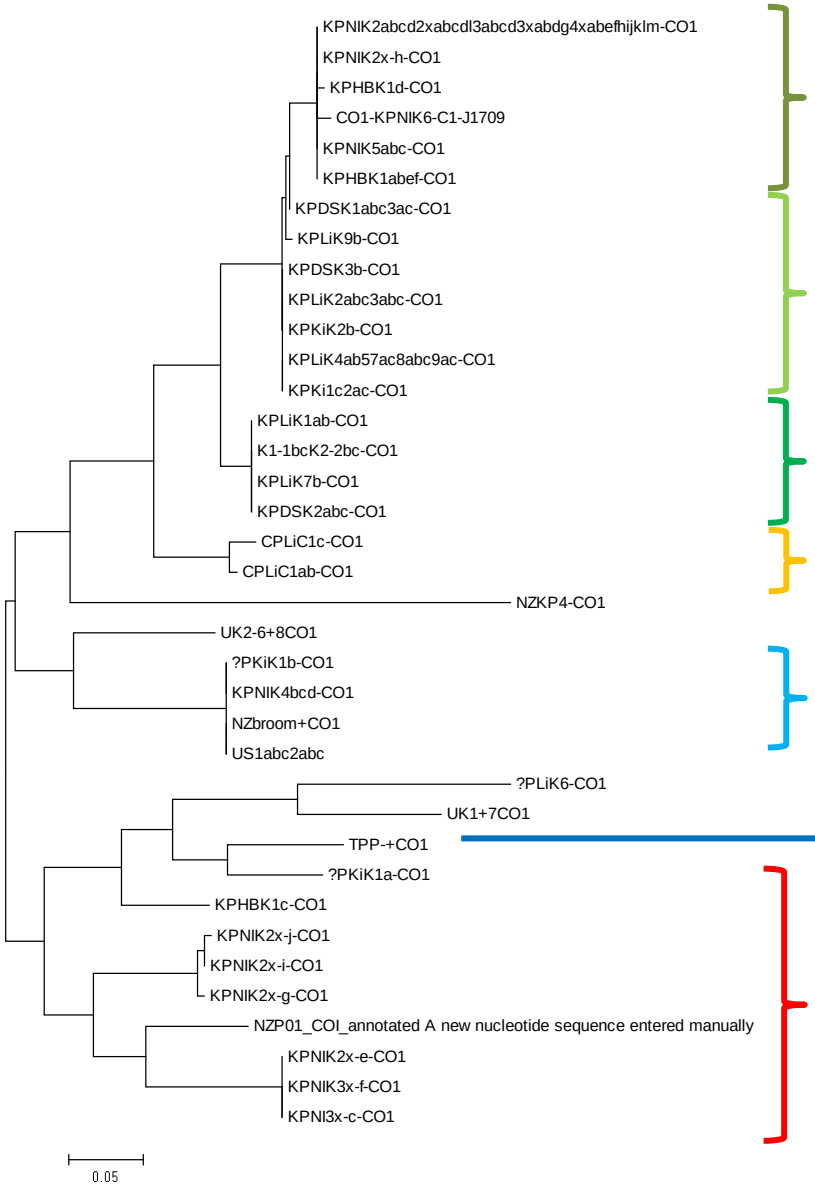
South Island kowhai psyllid B (*Psylla apicalis* ?)

Carmichaelia psyllid A and B?

NZ broom psyllid

TPP psyllid

Sequence data analysis



North Island kowhai psyllid (*Psylla apicalis* ?)

South Island kowhai psyllid A (*Psylla apicalis* ?)

South Island kowhai psyllid B (*Psylla apicalis* ?)

Carmichaelia psyllid A and B?

NZ broom psyllid

TPP psyllid

Other psyllids found on kowhai, e.g. acacia psyllid

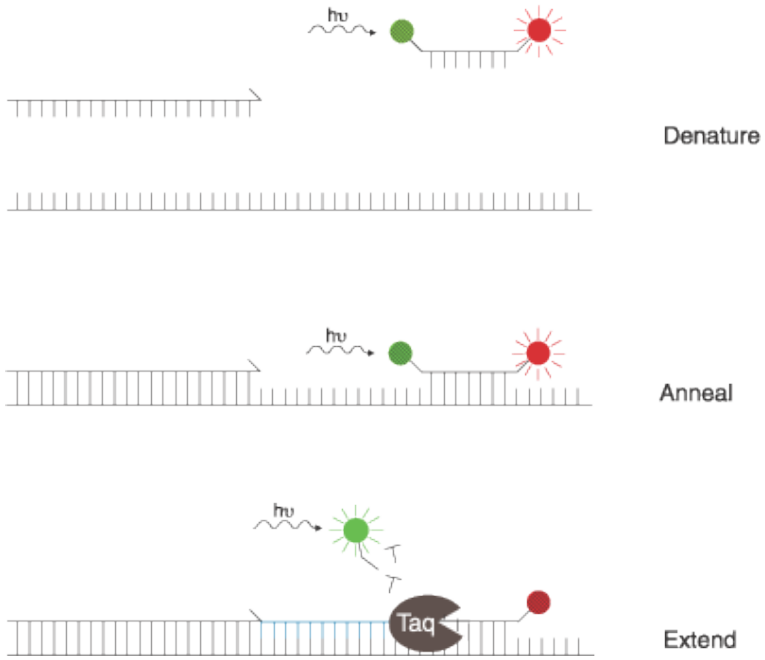
Approaching genetic diversity

- Sequence data analysis – psyllid species identification

- **Real-time PCR – Leu infestation testing of host and vector**

Real-time PCR analysis

Test for **presence or absence** of Leu and **quantitation**



http://www.bio.davidson.edu/Courses/Molbio/Molstudents/spring2003/Pierce/realtimepcr.htm

Results of first survey in NZ

Broom

- Leu is widespread over NZ
- Leu infestation occurs when psyllid population high
  - so does successful broom biocontrol
- Absence of broom psyllids correlates with absence of Leu
  - psyllids the exclusive vector
- Presence and quantity of Leu within sites highly variable

Kowhai and carmichaelia plant samples **Leu negative**

Non-broom psyllids Leu negative

Results of first survey internationally

- **Original collection sites in UK**
  - different broom psyllid species received
  - Leu negative
- **Californian samples**
  - same as NZ psyllid
  - Leu negative
  - broom psyllid with possibly low impact on invasive broom
- **Planning more sampling**
  - in NZ/Europe to confirm likely invasion route and
  - in California to understand biocontrol mechanism
- **New host-specificity testing + vector-specificity testing**

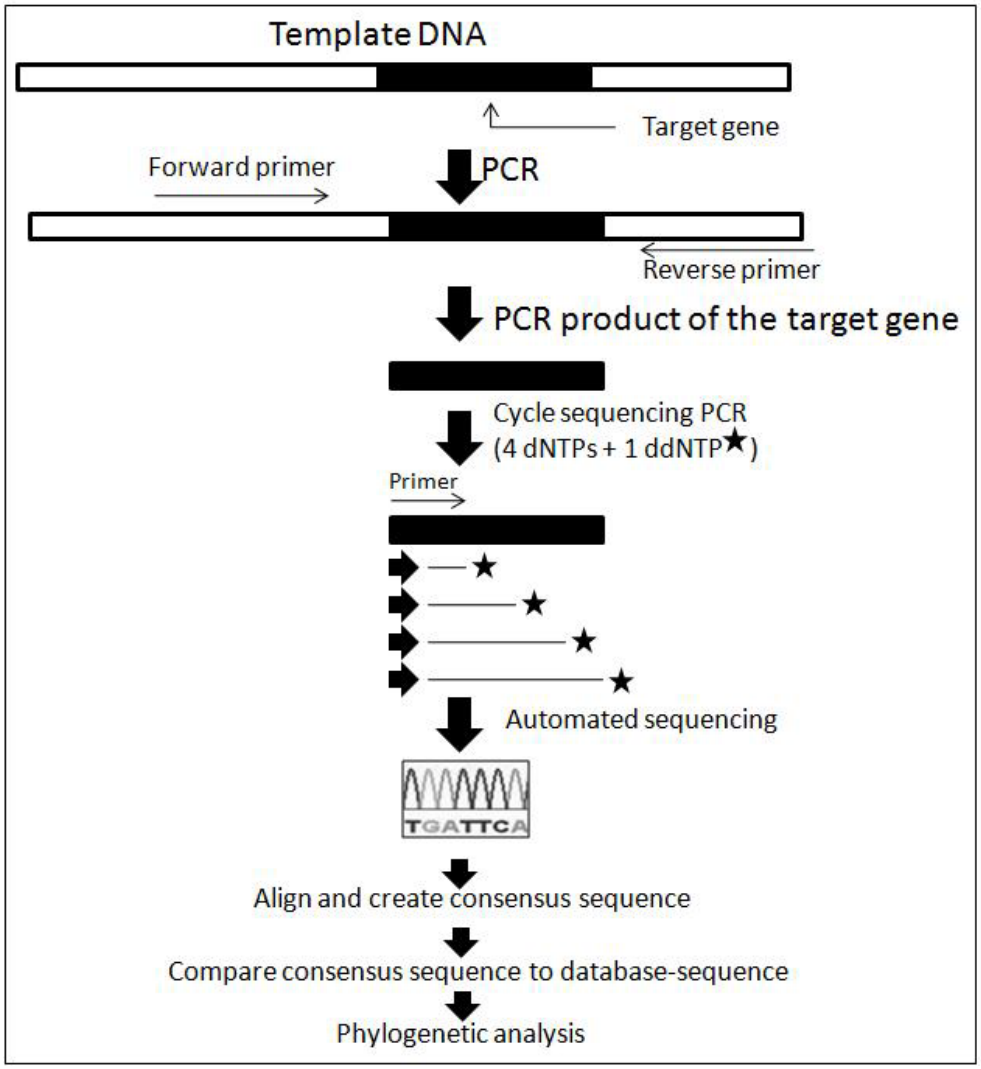
SUMMARY

- What is the origin of Leu?
- What is the role of Leu within broom biocontrol mechanism?
- What are distribution factors of Leu in NZ?
- Is it a biosecurity risk to native flora of NZ?
- Develop new risk assessment/testing protocols for the future

ACKNOWLEDGEMENTS

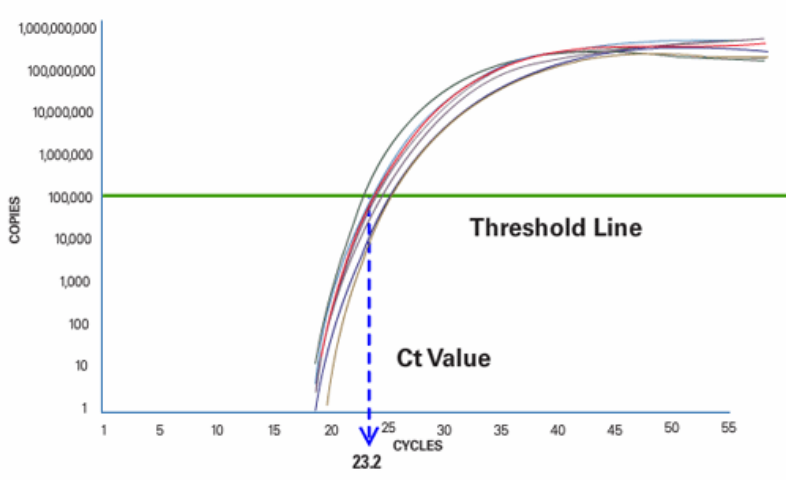
- Science + Innovation Group in the **Ministry of Business Innovation and Employment** (Beating Weeds Project: Contract CO9X0210)
- Simon Fowler (**Managing Invasive Weeds, Pest & Diseases**), Gary Houliston (**Biodiversity & Conservation**)
- Andrew Pitman, Sam Beard – **Plant & Food Research**
- Chris Winks (**LCR**), Lia Liefting (**MPI**), Jenny Dymock (**Northland Regional Council**), Randall Milne (**Env. Southland**), Dick Shaw (**CABI, UK**), Brian Hogg (**Uni. California**)
- Sequencing Team LCR Tamaki

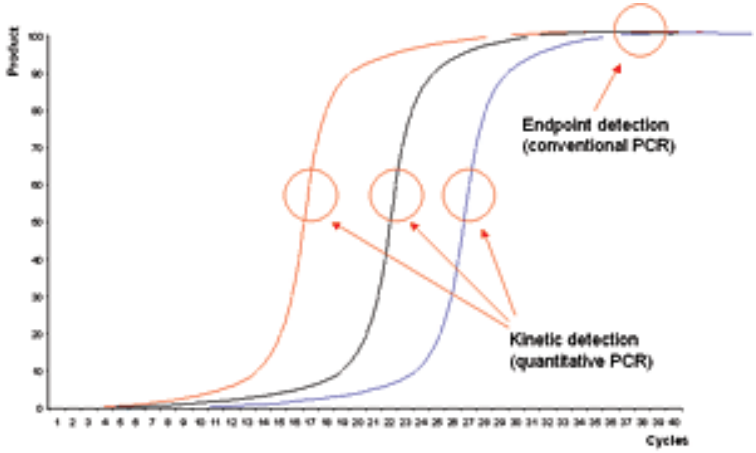
DNA sequence data



Real-time PCR analysis

Quantitation:
certain amount of fluorescence achieved after x amplification





http://www.ebioservice.com/eng/show_news.asp?id=506
http://www.eppendorf.de/int/index.php?l=211&action=products&contentid=99&sitemap=2.5.1.5

Results of pre-liminary survey in NZ

Broom collection Location	Psyllids present	Leu present in broom psyllid	Leu present in broom stem
Rotorua	No	n/a	5/5 negative
Turangi South	Yes	8/17 positive	3/5 positive
Taupo	partially	8/17 positive	5/5 negative
Waihi Town / Beach	No	n/a	5/5 negative
Kuratau	Yes	6/24 positive	5/5 negative
Lichfield	No	n/a	5/5 negative
Waihiti	Yes	5/5 negative	5/5 negative
10 sites around Lincoln	Yes	24/30 positive	7/10 positive