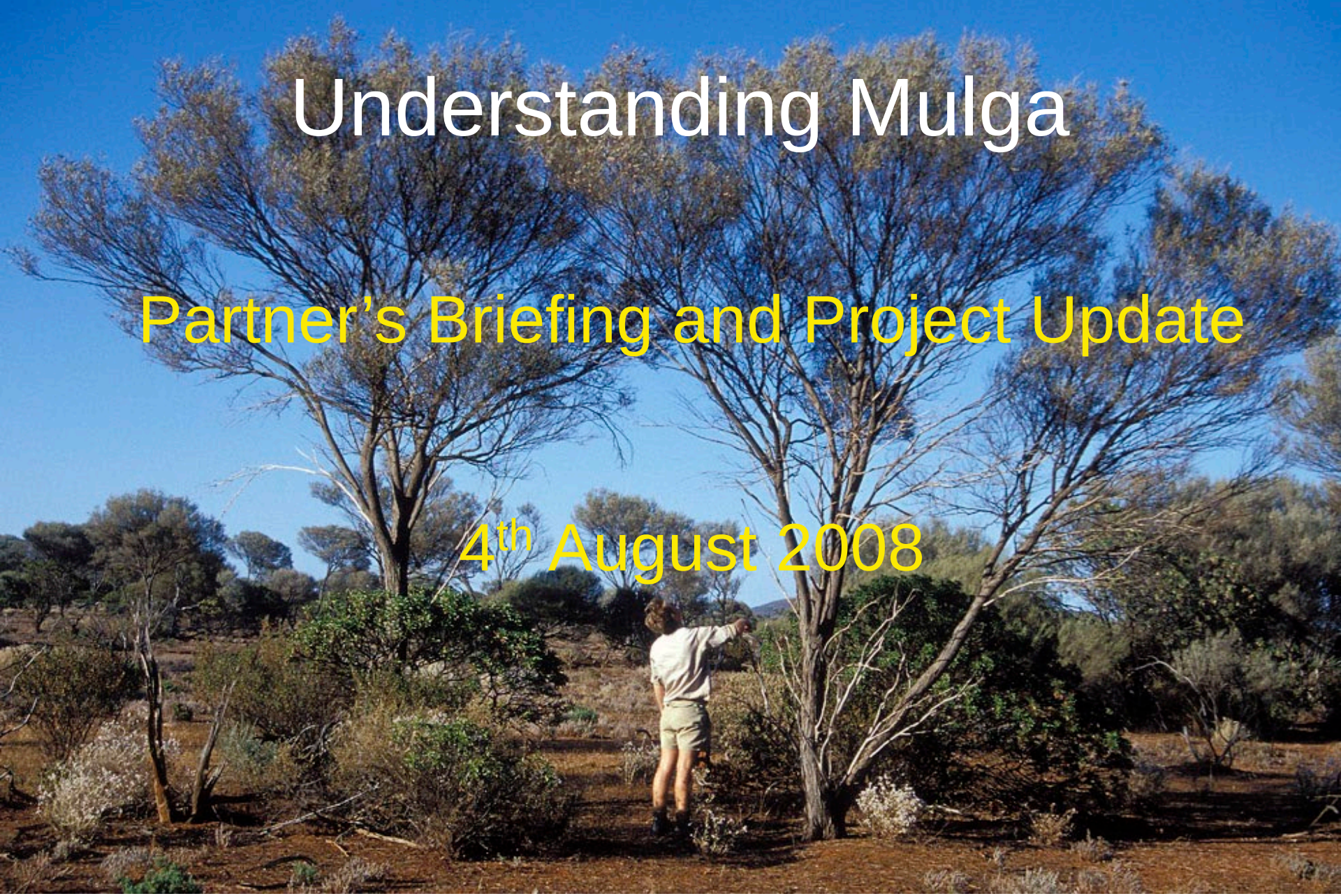


Understanding Mulga

Partner's Briefing and Project Update

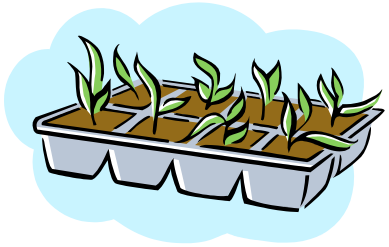
4th August 2008

bhpbilliton RIO TINTO FMG ACT NOW FOR THE FUTURE Department of Environment and Conservation
Our environment, our future

Agenda

- Housekeeping: Stephen van Leeuwen
- Background: Stephen van Leeuwen
- Field studies & Taxonomic evaluation: Bruce Maslin
- Ontogenetic (seedling) study: Jordan Reid
- Genetic studies: Joe Miller
- Way forward: All





Understanding Mulga

Project Background

bhpbilliton RIO TINTO FMG ACT NOW FOR THE FUTURE Department of Environment and Conservation

Our environment, our future

Objective

To elucidate patterns of variation within Mulga in order to provide a reliable means of identifying the types present.

Primary Drivers

- Poor discrimination of Mulga types in WA
- High diversity of Mulga types in Rangelands (Eastern Hamersley Range & North Eastern Goldfields)
- High mineral prospectively in Mulga diverse landscapes
- Poor understanding of distribution & conservation status of Mulga types (SRE taxa, EIA, Risk matrix)
- Knowledge impediments for land management (fire) and sustainable use (rehabilitation/restoration)

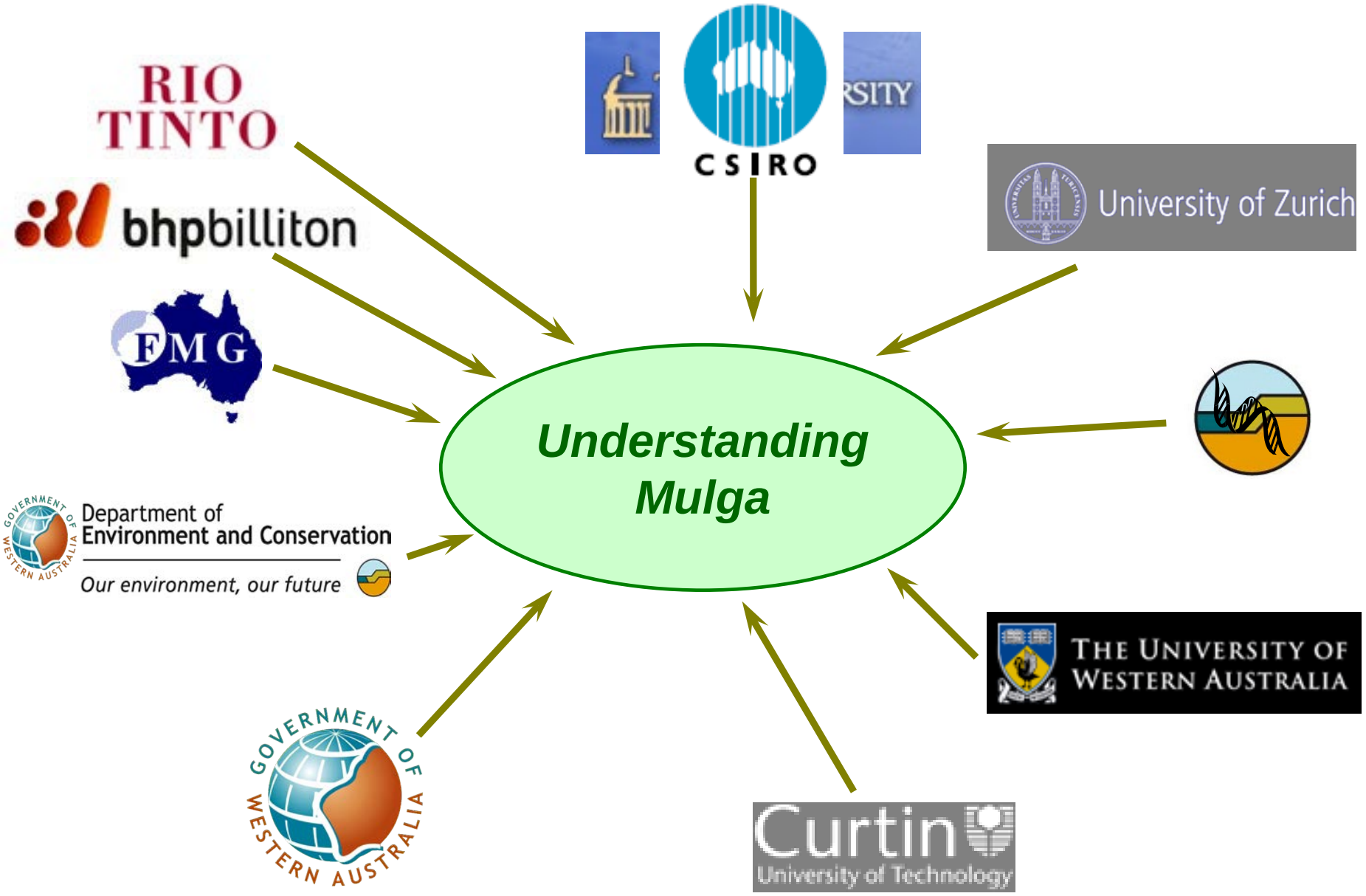
Outputs - Direct

- Comprehensive taxonomic review of Mulga
- Determination of the genetic factors responsible for Mulga diversity
- Assessment of the conservation status of Mulga types
- Electronic key to identify Mulga types
- Website for *Understanding Mulga* project
- Scientific publications (taxonomic & genetic)
- *Mulga Manual* (hardcopy & web)

Outputs - Derived

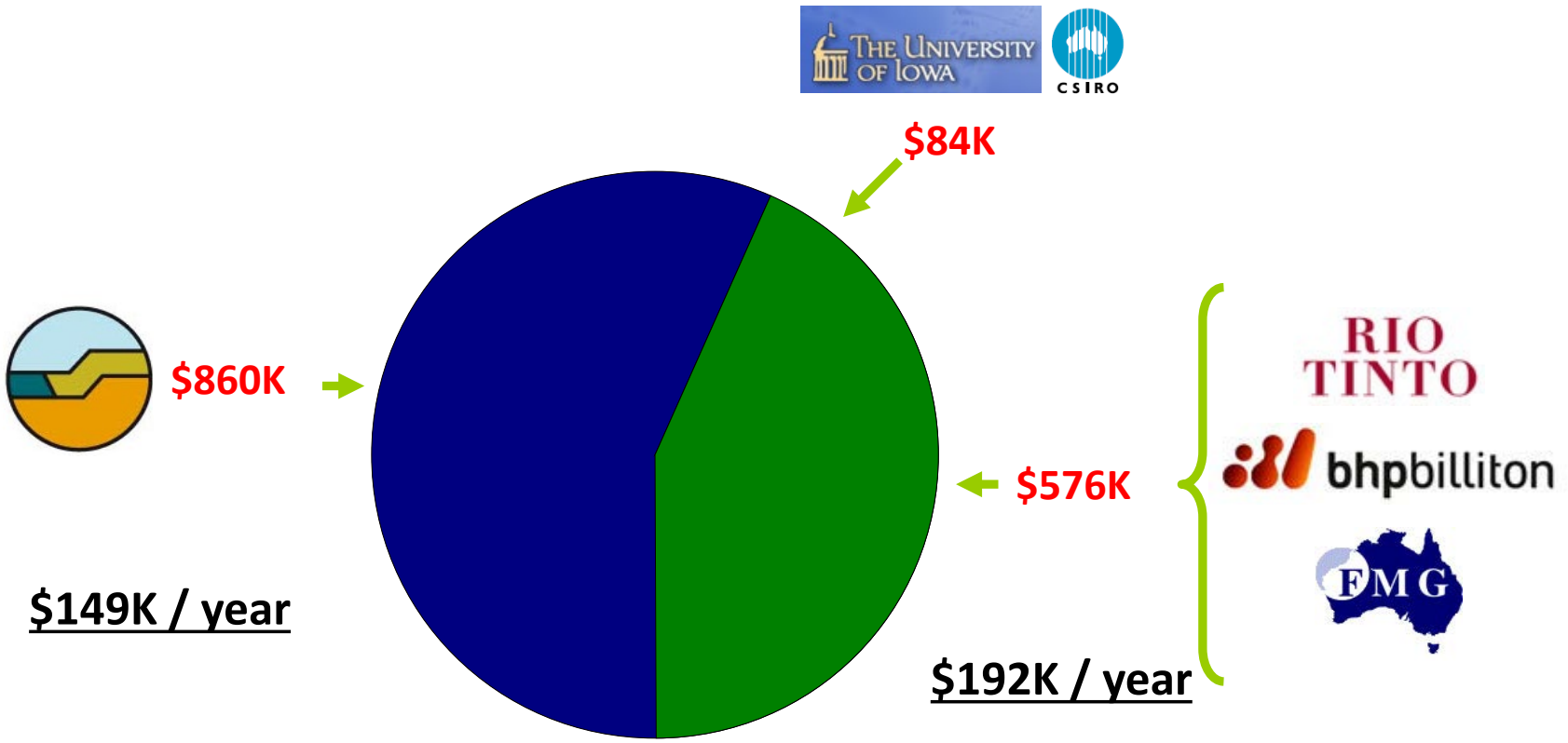
- Information to assist with the management, conservation & utilisation of Mulga (species & communities)
- Provide basis for understanding Mulga over its entire geographic range
- A framework for undertaking & interpreting applied ecological studies involving Mulga

The Project – A Partnership



Project Budget

- Three Year Project
- Total Budget **$1 520 000**



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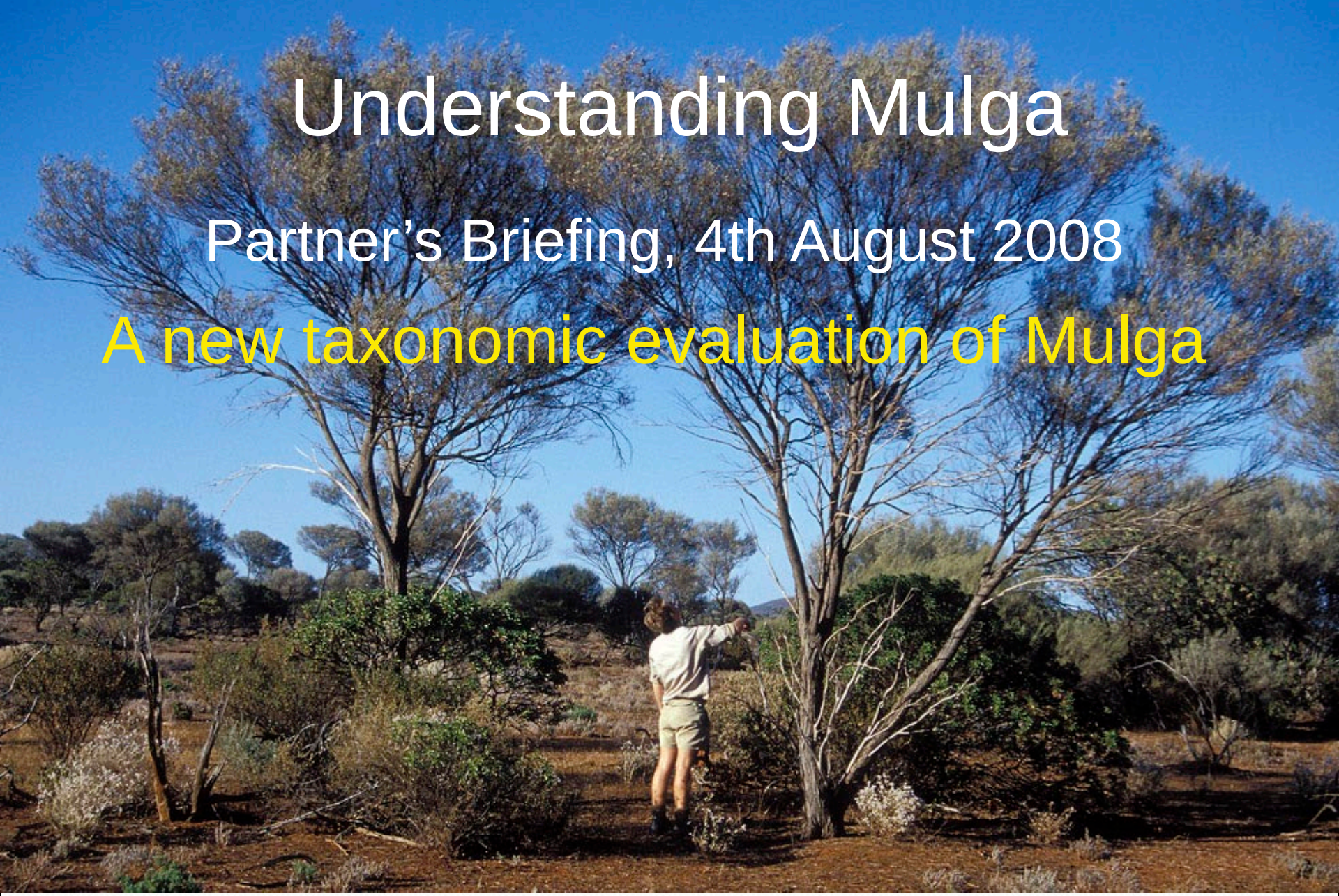
Understanding Mulga

Partner's Briefing, 4th August 2008

A new taxonomic evaluation of Mulga



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A new taxonomic evaluation of Mulga

- Background information on Mulga.
- Field study report.
- Taxonomic findings.
- Electronic key.

What is Mulga?

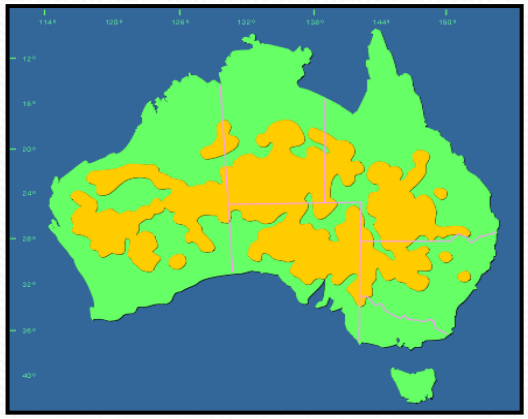

Acacia aneura


Near Meekatharra

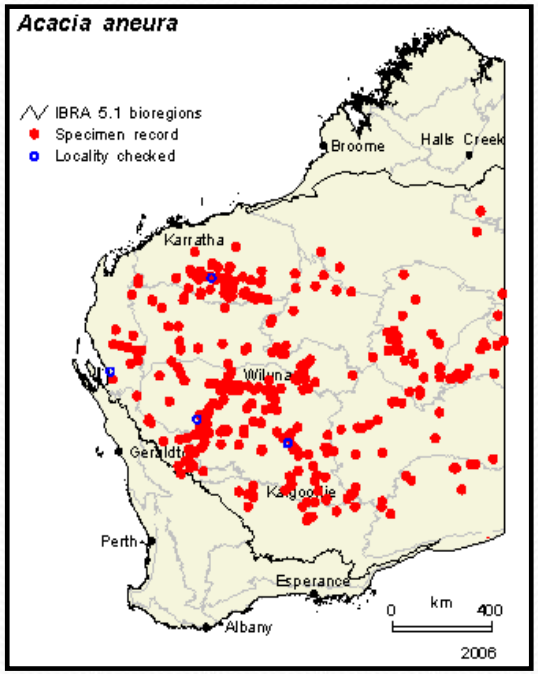

A Mulga community


Near Lake Austin

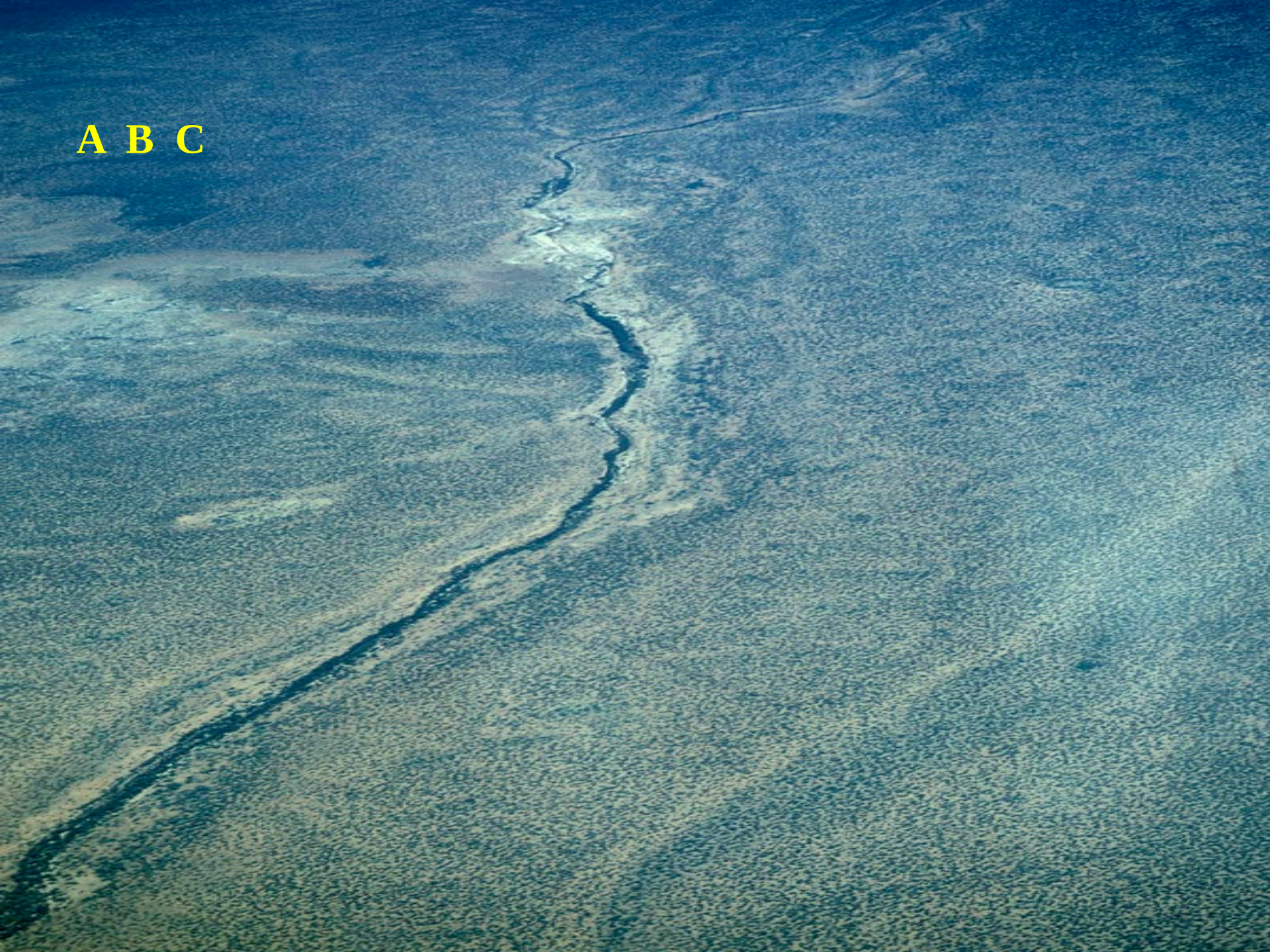
The Mulga group:
some characteristics



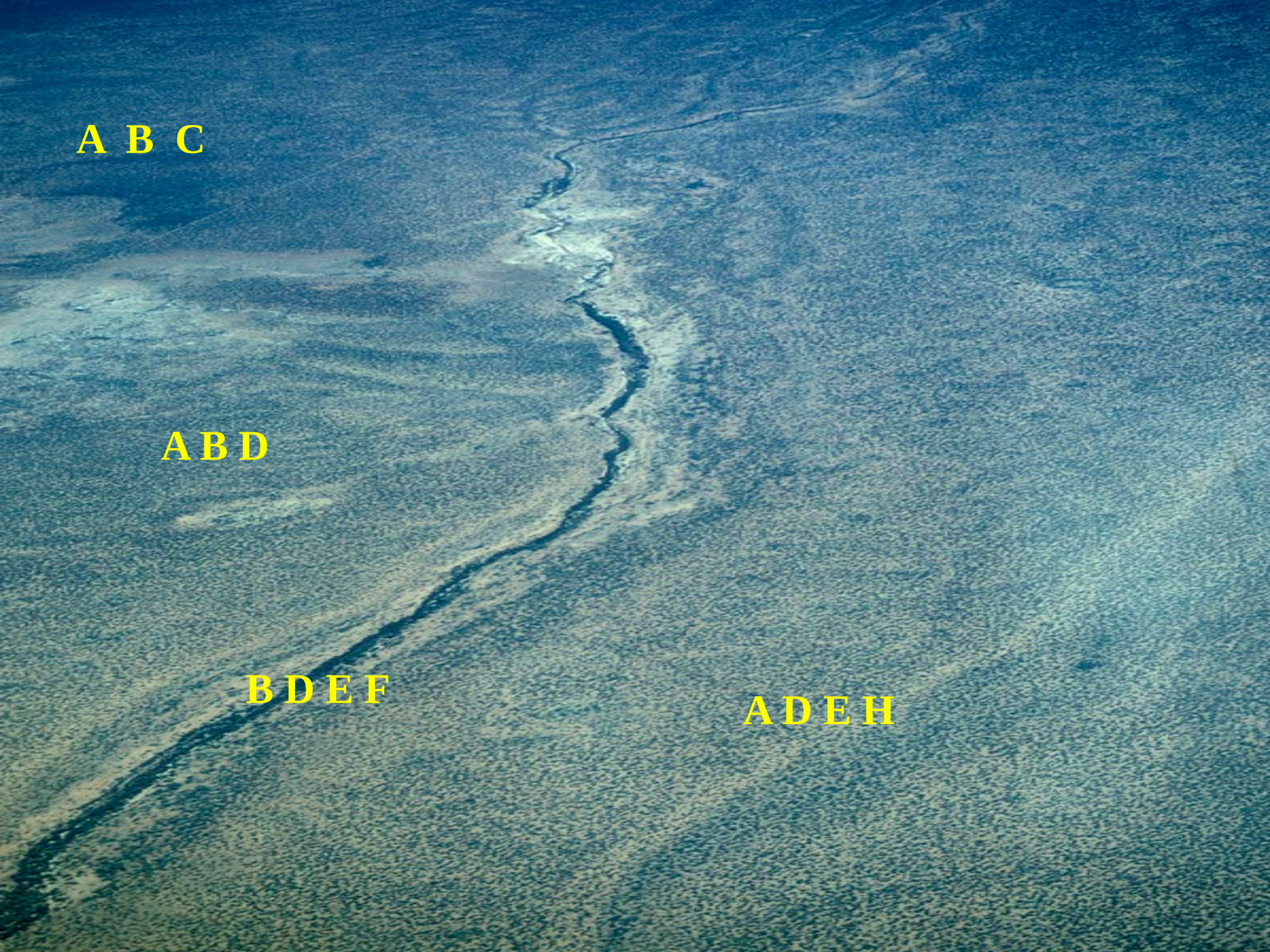
- Occupies 20% of Arid Australia.
- Highly variable complex (about 20-30 different morphotypes).
- Taxonomically the most difficult group of Acacia in Australia.
- Taxonomy largely unresolved (despite Randell 1992; Pedley 2001).
- Variation occurs both within and between populations (intra-population variation is especially conspicuous).
- Populations form a complex mosaic across landscape.

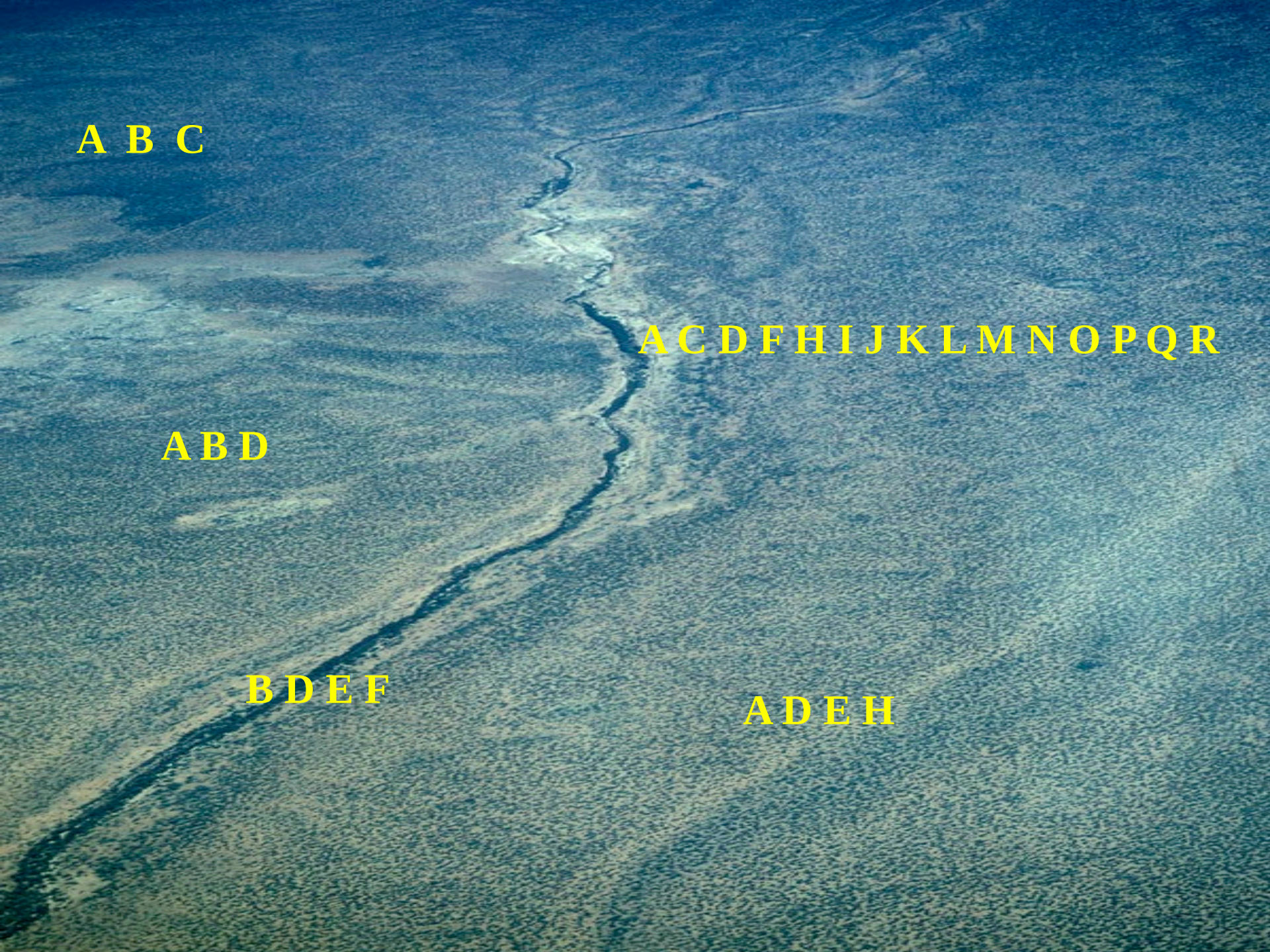



Mulga land near Lake Austin

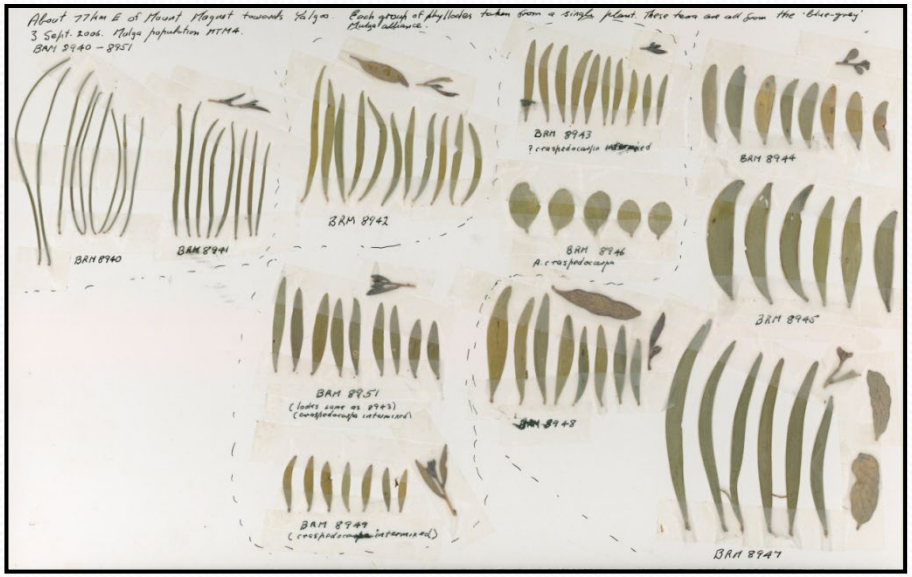

A B C


A B C
A B D


A B C
A B D
B D E F
A D E H


A B C
A C D F H I J K L M N O P Q R
A B D
B D E F
A D E H

Intra-population variation in Mulga



The Mulga Complex

Mulga core species

- *Acacia aneura* (Mulga: 10 varieties)
- *Acacia ayersiana* (Broad leaf Mulga)
- *Acacia minyura* (Shrubby desert Mulga)
- *Acacia paraneura* (Weeping Mulga)

Mulga close relatives

- *Acacia craspedocarpa* (Hop Mulga)

The Mulga group

Mulga allied species

- *Acacia atopa*
- *Acacia brachystachya* (Turpentine Mulga)
- *Acacia catenulata* (Bendee)
- *Acacia clelandii* (Cleland's Mulga)
- *Acacia coolgardiensis* (Sugar Brother) and allies
- *Acacia ramulosa* (Horse Mulga, Bowgada) & allies
- *Acacia substessaragona*
- *Acacia thoma*

Mulga core group species:

A. aneura (some varieties)

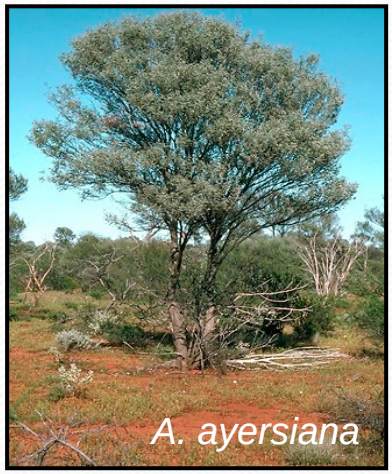

A. aneura var. *pilbarana*


A. aneura var. *argentea*


A. aneura var. *microcarpa* (adolescent plant)


A. aneura var. *conifera*

Mulga core group species


A. ayersiana


A. ayersiana


A. ayersiana


A. minyura


A. paraneura

Mulga close relatives:

A. craspedocarpa (Hop Mulga)

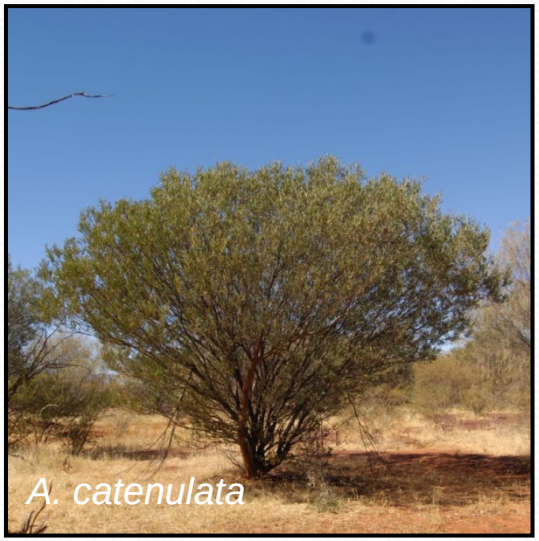
Mulga close relatives:

A. craspedocarpa (Hop Mulga)

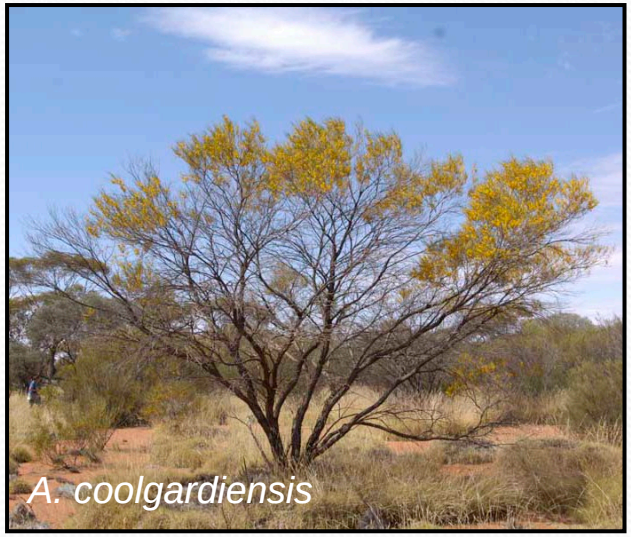
Mulga allied species


A. ramulosa


A. atopa


A. catenulata


A. ramulosa


A. coolgardiensis

Aims of the *Understanding Mulga* project

- Identify different Mulga types (W.A.); analyse variation patterns; describe 'good' taxa. (Field & herbarium studies)

- Determine factors responsible for creating and maintaining variation. (Genetic & ontogenetic studies)

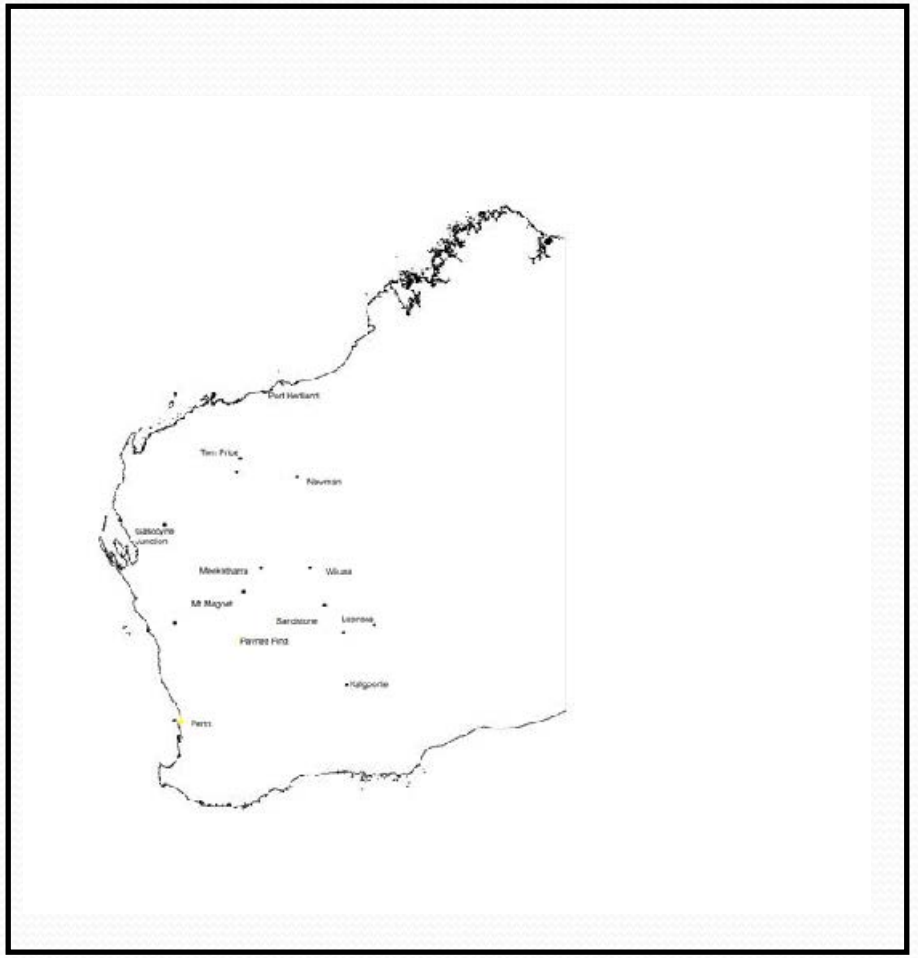
Clearly defined (and named) taxa are essential for effective management, conservation & utilisation of Mulga

- Provide means to identify Mulgas (Lucid key); deliver relevant information concerning the taxa (Mulga Manual, website, publication).

Field Studies

2006 – 2007

- 4 trips
- 673 herbarium collections
- 1337 genetic collections
- 140 seed collections



2008/2009

- W.A., N.T. & S.A.

Constraints

- Large distances (selective sampling)
- Dry seasons (poor pod crops)

Taxonomic studies:
Important characters

- Pods
- Branchlets (ribs and resin)
- New shoots (colour, hairs, resin)
- Phyllodes (size, shape, curvature, cross-section)
- Gland position
- Flowers (?)

Pod wings

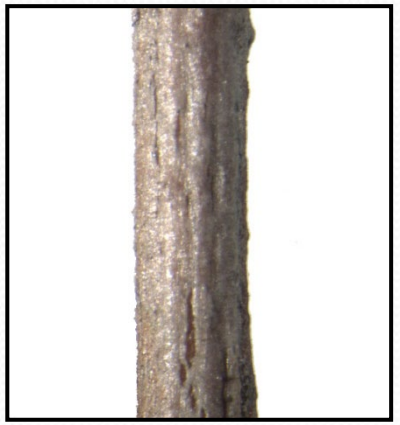
Pod wings

Branchlet resin

Young branchlet

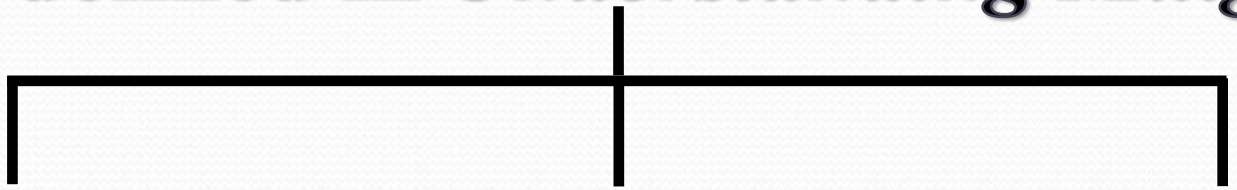
Penultimate branchlet

Mature branchlet



Mulga group Alliances

(as defined in *Understanding Mulga*)



Green

Pods not winged

Branchlet resin translucent

Blue

Pods winged

Branchlet resin opaque

Grey-Green

Pods winged

Branchlet resin translucent

Mulga group Alliances
(as defined in *Understanding Mulga*)

Green

Pods not winged

Branchlet resin translucent

Blue

Pods winged

Branchlet resin opaque

Grey-Green

Pods winged

Branchlet resin translucent

Pedley (2001)	***Understanding Mulga* (2008)**
• var. *macrocarpa*	• var. *macrocarpa*
	• var. *macrocarpa* (flat phyllode)
• var. *pilbarana*	• var. *pilbarana*
• var. *tenuis*	• var. *tenuis*
	• var. *tenuis* (flat)

Acacia aneura: Green alliance


A. aneura var. *pilbarana*

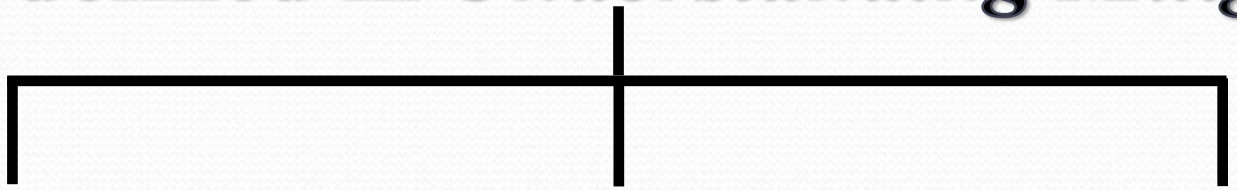

A. aneura var. *tenuis*


A. aneura var. *macrocarpa*


A. aneura var. *tenuis*


A. aneura var. *macrocarpa*

Mulga group Alliances
(as defined in *Understanding Mulga*)



Green

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Branchlet resin translucent

Blue

Pods winged

Branchlet resin opaque

Grey-Green

Pods winged

Branchlet resin translucent

Pedley (2001)	***Understanding Mulga* (2008)**	
• var. *argentea*	• var. *argentea*	• *A. ayersiana*
	• var. *argentea* (narrow phyllode)	• *A. minyura*
	• var. *argentea* (short phyllode)	• *A. aff. minyura* (Hilltop variant)
• var. *microcarpa*	• var. *microcarpa*	
	• var. *microcarpa* (thick resin)	
	• var. *microcarpa* (broad incurved phyllode)	
	• var. *microcarpa* (broad recurved phyllode)	

Acacia aneura: Blue alliance


A. aneura var. *argentea*


A. aneura var. *argentea*


A. aneura var. *microcarpa*


A. aneura var. *argentea*


A. aneura var. *microcarpa*

Mulga group Alliances
(as defined in *Understanding Mulga*)

Green

Pods not winged

Branchlet resin translucent

Blue

Pods winged

Branchlet resin opaque

Grey-Green

Pods winged

Branchlet resin translucent

Pedley (2001)

- var. *fuliginea*
- var. *intermedia*

***Understanding Mulga* (2008)**

- var. *fuliginea*
- var. *fuliginea* (narrow phyllode)
- var. *intermedia*
- var. *intermedia* (linear)
- var. *intermedia* (resinous)
- var. *alata*
- var. *alata* (narrow phyllode)
- var. *alata* (narrow pod)
- var. *alata* (pseduo-winged)
- *A. craspedocarpa*
- *A. paraneura*

Acacia aneura: Grey-green alliance

A. aneura var. *intermedia*

A. aneura var. *intermedia*

A. aneura var. *fuliginea*

An example..

***A. aneura* var. *alata* (narrow phyllode variant)**

(53 specimens at W.A. herbarium)

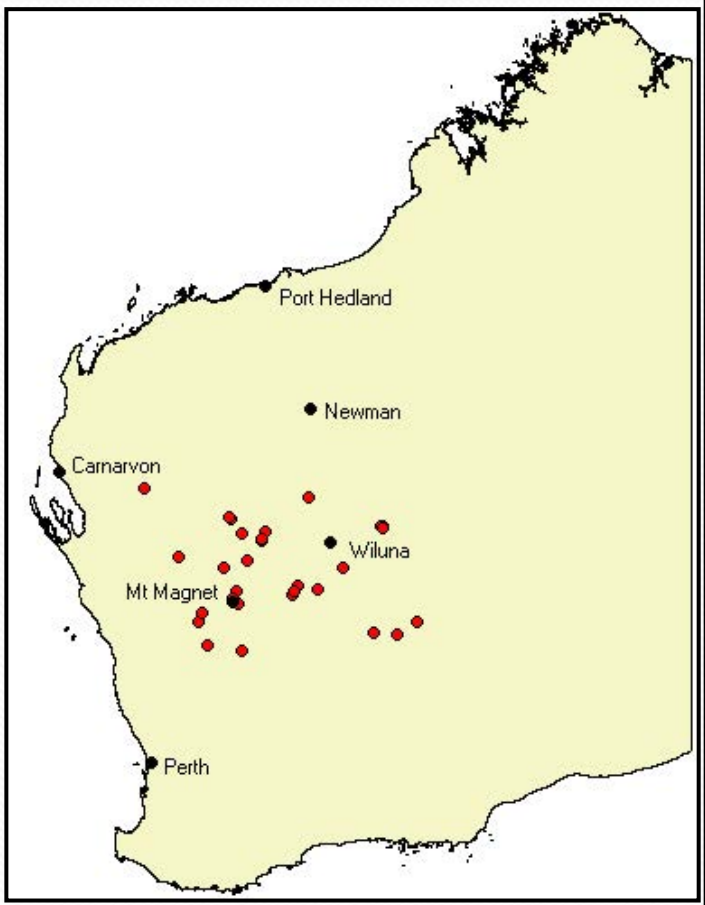
Names applied by Pedley to these specimens;

A. aneura var. *aneura*

A. aneura var. *microcarpa*

A. aneura var. *tenuis*

A. paraneura



Lucid Key (demonstration)

Summary of taxonomic findings

Characters

- Pod and vegetative characters reviewed; better understanding developed (esp. pod wings: anatomy in progress)
- New characters identified (e.g. branchlet resin)
- Cluster of vegetative characters recognized (assist in identification of sterile material)

Taxa

- Elucidation of major groupings (Green, Blue and Grey-green alliances)
- 22 W.A. Mulga taxa currently recognized (formerly 7 taxa); validation/verification of taxa progressing
- Methods developed for recognizing hybrids

Key

- Preliminary draft constructed

Take home messages

- Mulga - large, variable and complex group
- Our broad conceptual framework (Blue, Green & Grey-green Alliances) is novel and a major achievement
  - Will form basis for future studies
- The taxonomic process is very time-consuming
  - Find characters/verify taxa
- Critically important to sample all morphotypes in Mulga populations
  - Many specimens/genetic samples
- Mulga populations are geographically widespread over the landscape
  - Logistically challenging to sample
- I am confident we have the expertise to meet the challenge of resolving complexities in Mulga (given sufficient time)!

The taxonomic process

Populations

Analyze variation

- Field studies
- Morphological studies
- Genetic studies
- Other studies (ontogeny, anatomy)

Species defined

Species named

Assemble information about species

- Distribution
- Description (morphology)
- Biological data
- Images

Distribute information about species

- Mulga Manual
- Research papers
- Recovery plans
- Conservation status

- Threatening processes
- Salinity control strategies
- Flora management

Understanding & defining variation in Mulga is crucial to its effective conservation & utilisation.

Understanding Mulga

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Taxonomic studies:

The Mulga complex

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Mulga close relatives

- *Acacia craspedocarpa* (Hop Mulga)

The Mulga group

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- *Acacia ramulosa* (Bowgada) and allies
- *Acacia substessaragona*

Understanding Mulga

Partner's Briefing, 4th August 2008

An ontogenetic (seedling) study of Mulga

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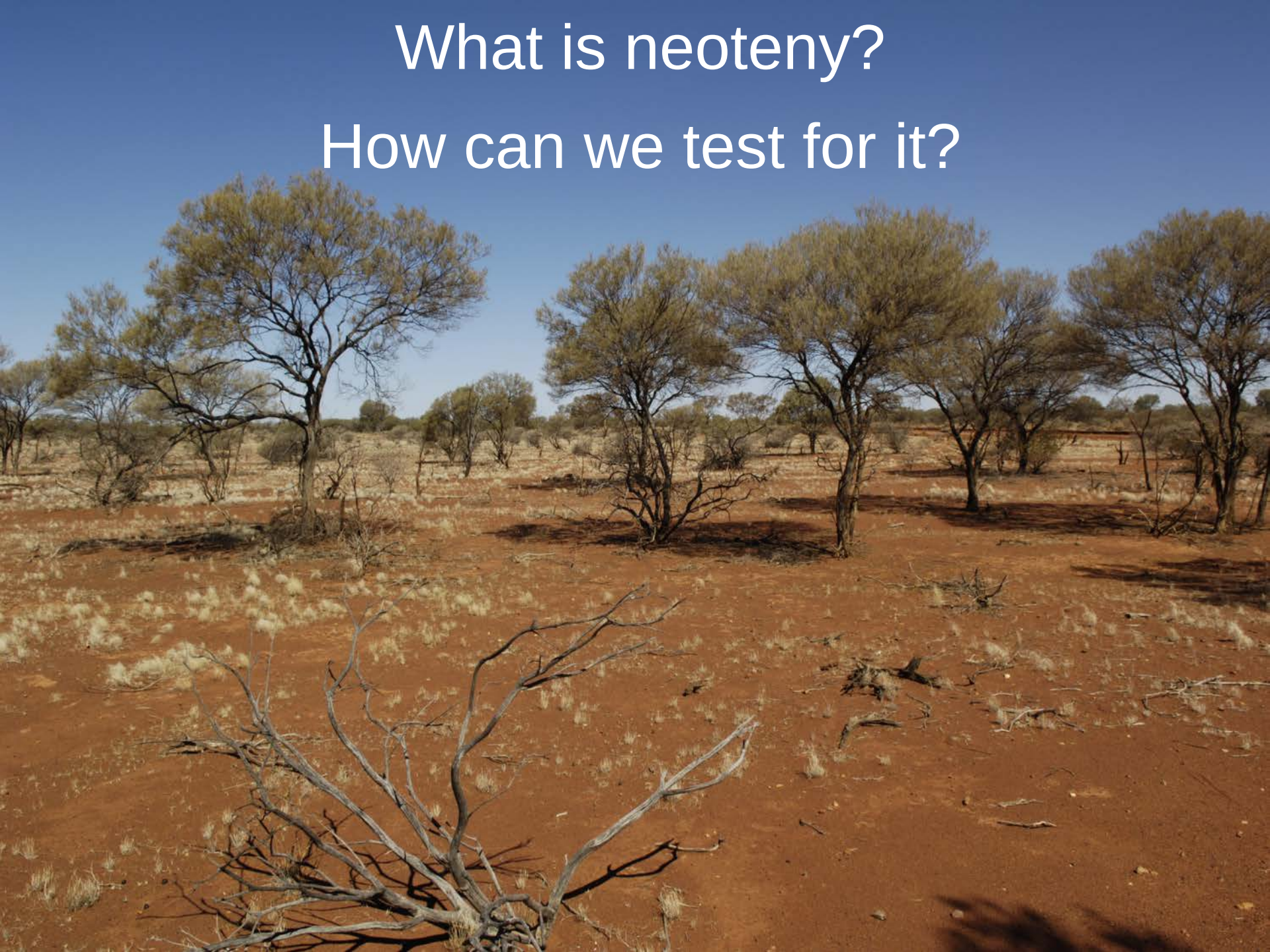
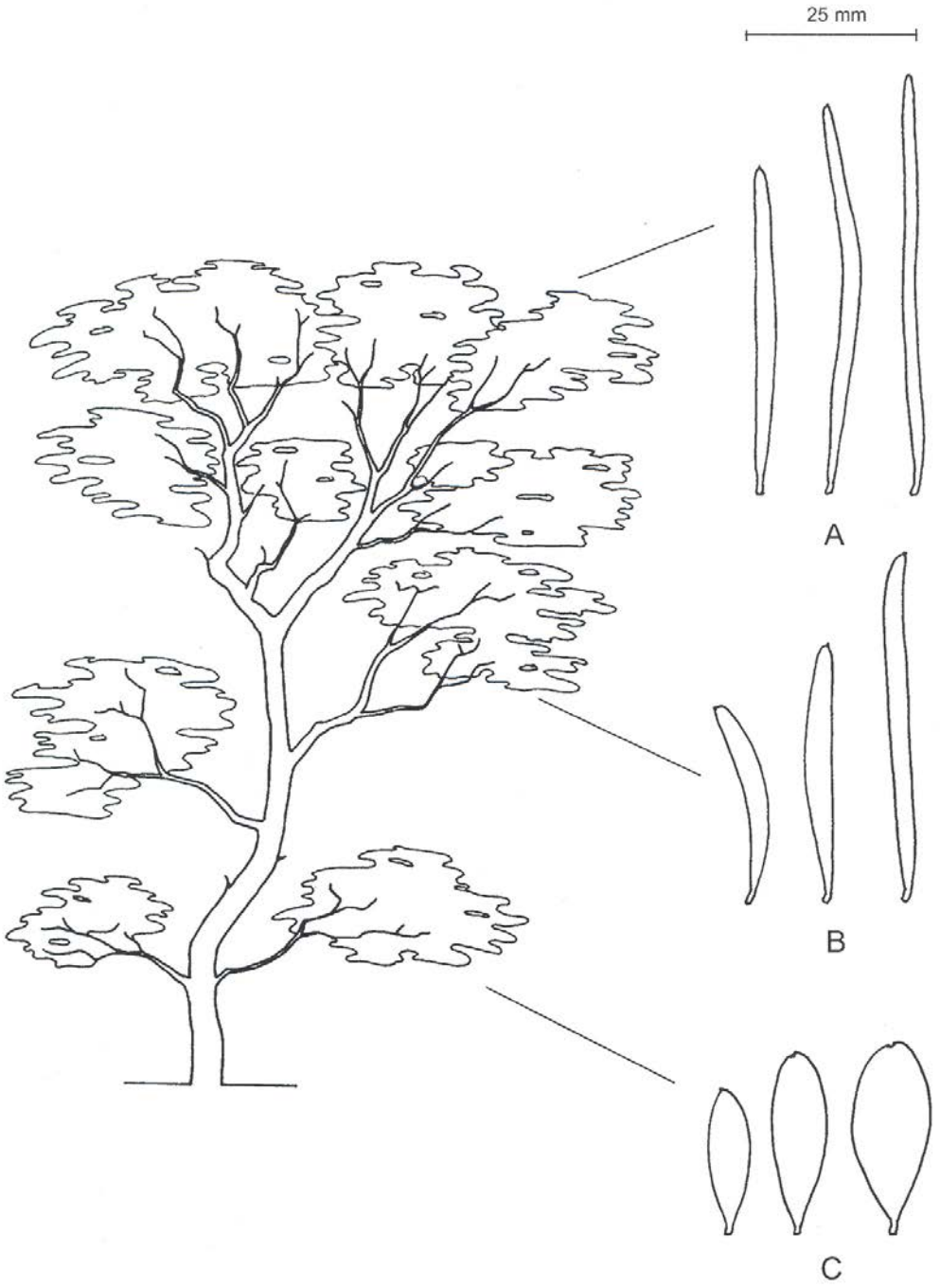
What is neoteny?

How can we test for it?



Neoteny is the retention of juvenile features in the adult growth phase.

It is important to note that neotenous plants can still flower and fruit.



1. Examine populations in the field

2. Genetically sample plants

3. Conduct seedling studies

Why are we undertaking this study?

We want to determine if;

- neoteny is commonly present in W.A. Mulga populations?
- neoteny occurs, is it present in all three Mulga alliances?

Based on current evidence we hypothese that neoteny is most prevalent in the blue alliance of Mulga.

Constraints to this study

The largest and most important constraint is our ability to collect seed.

Our ability to successfully germinate seed of the blue alliance taxa.

Having sufficient time to grow & adequately document the seedling growth phases.

Methodology

Mulga seed collections.

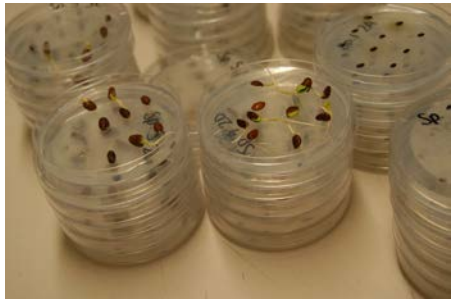
Seed pre-treatments experiment.

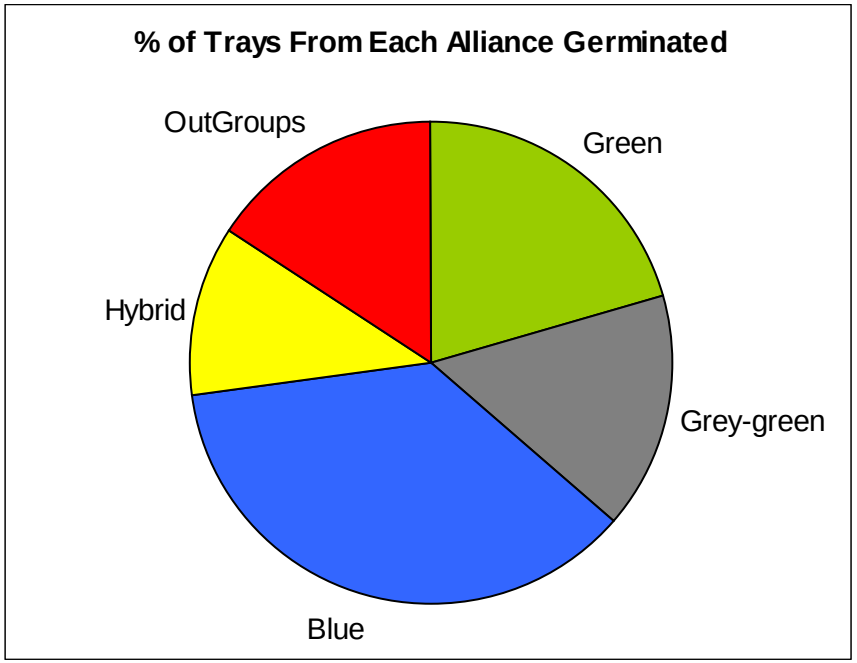
Germination of seed collections.

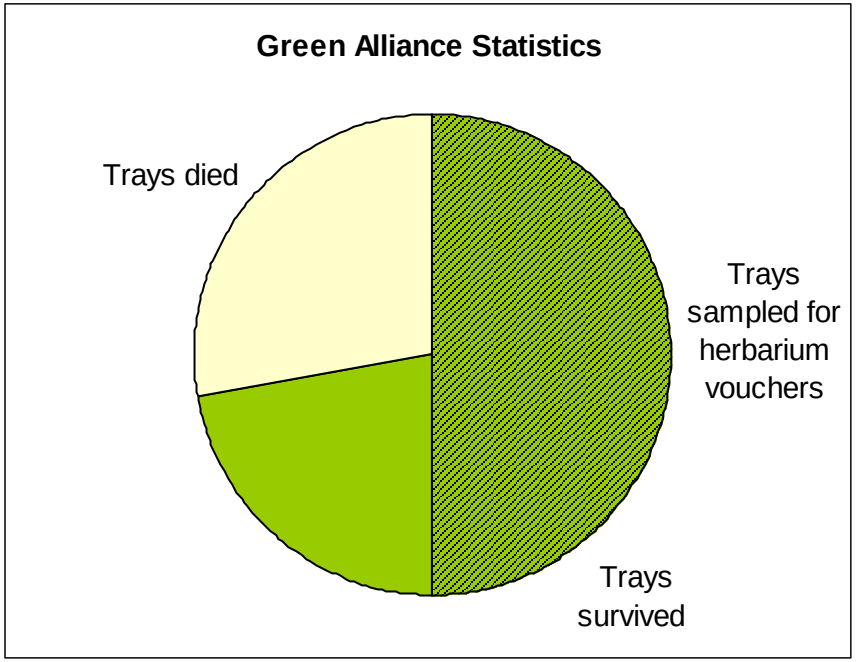
Monitoring of seedlings and data recording.

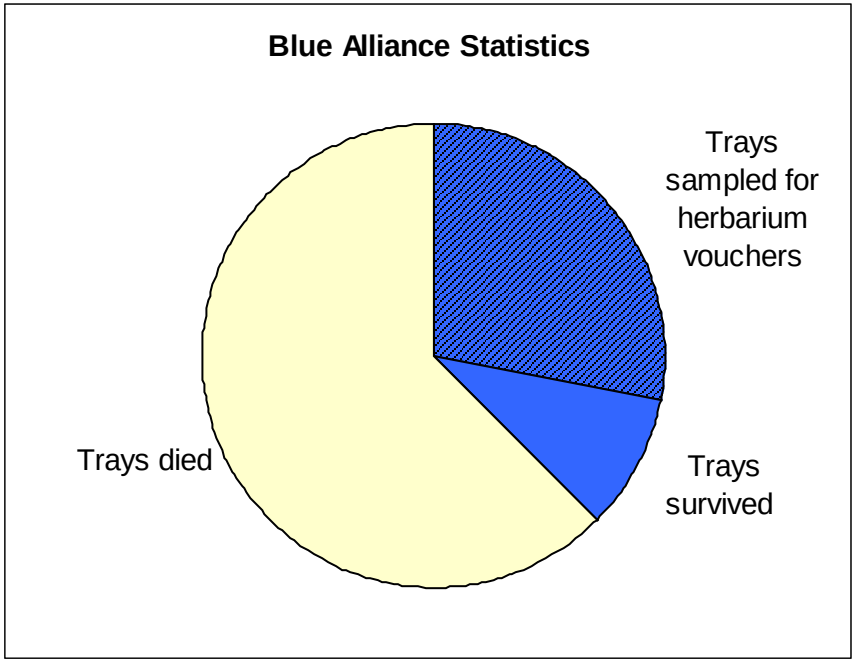
Destructive sampling for herbarium vouchers.

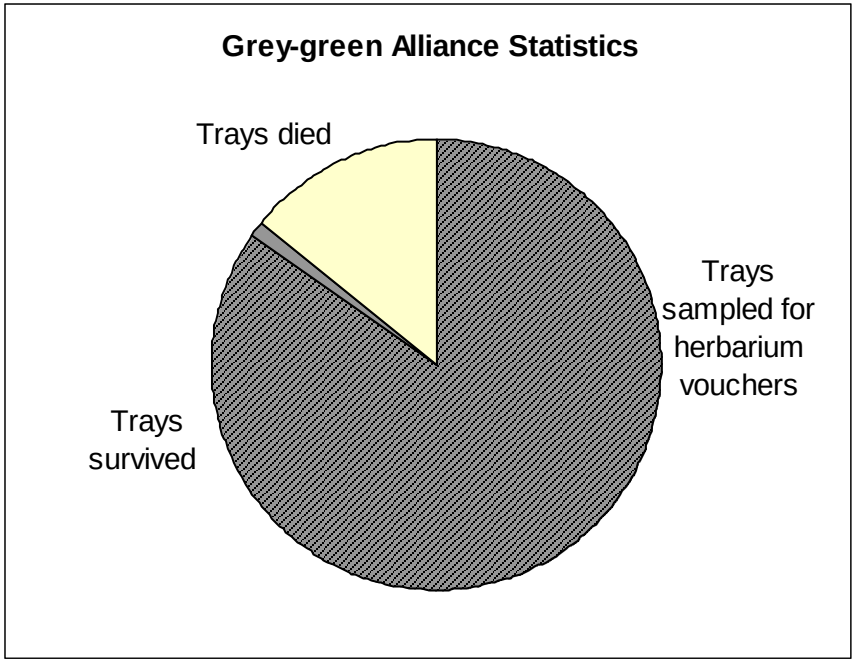
Planting out at Curtin University field trial area.





% of Trays From Each Alliance Germinated
OutGroups
Green
Hybrid
Grey-green
Blue


Green Alliance Statistics
Trays died
Trays sampled for herbarium vouchers
Trays survived


Blue Alliance Statistics
Trays sampled for herbarium vouchers
Trays died
Trays survived


Grey-green Alliance Statistics
Trays died
Trays sampled for herbarium vouchers
Trays survived

A. aneura var. tenuis (Green alliance)

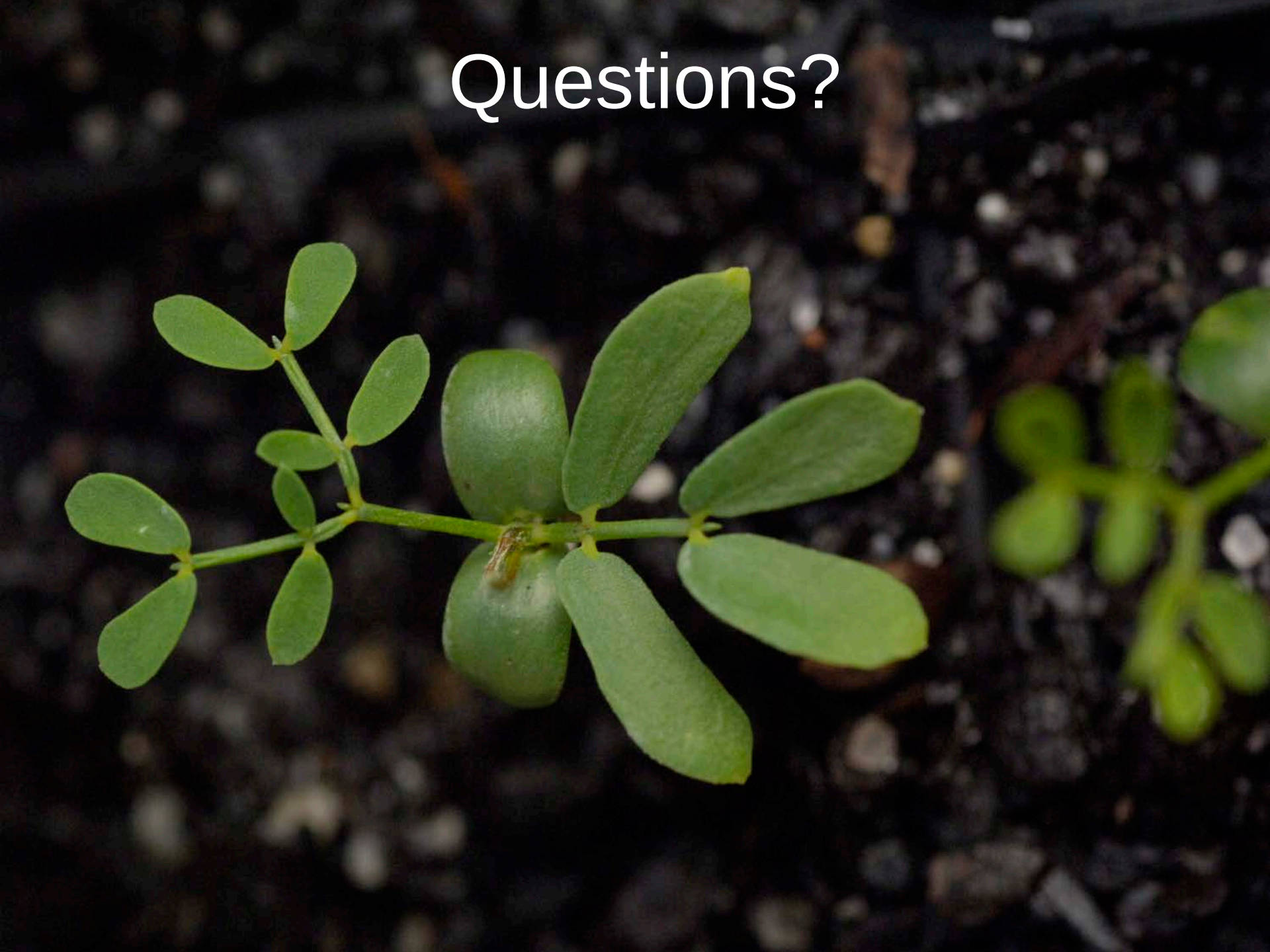
Adult foliage showing round phyllodes

Juvenile foliage showing flat phyllodes

Where do we go from here?

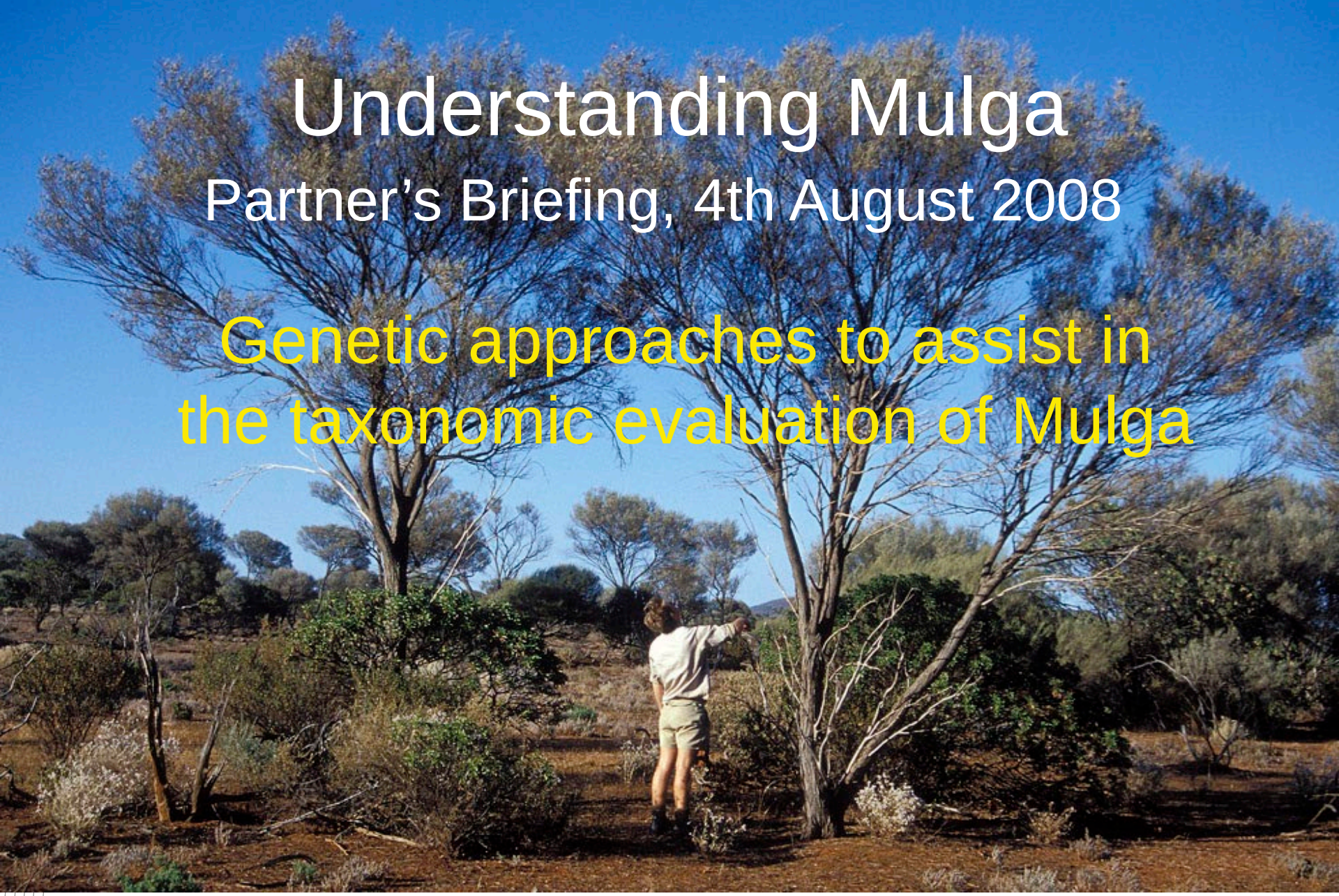
- Fill in the gaps in our knowledge.
- We learn from experience.
- We will germinate more seed and collect more data.
- We will critically examine our results.

Questions?



Understanding Mulga

Partner's Briefing, 4th August 2008



Genetic approaches to assist in the taxonomic evaluation of Mulga

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Genetic Data

- The comparison of DNA sequences among plants to infer evolutionary relationships
- Use of the genetic data can confirm morphological inferences and can provide data where morphology is equivocal

Genetic Data

- The Taxonomic approach mainly uses morphological data
- Genetic and taxonomic data are complementary
- Genetics can provide explanations for the morphological variation
- They may give conflicting answers
  - Convergent evolution
  - Hybridization
- Not finding the genes responsible for traits!
- Studying the signature of evolution in the DNA
  - Neutral evolution

Goals of Genetic work

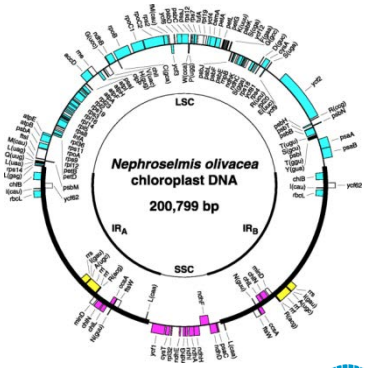
- Use DNA sequence information to determine how plants are related to each other
- Use DNA sequence information to determine how **groups of plants (species)** are related to each other
- Infer the factors producing and maintaining the morphological variation
- Facilitate the identification and management of Mulga

Factors Producing and Maintaining Variation

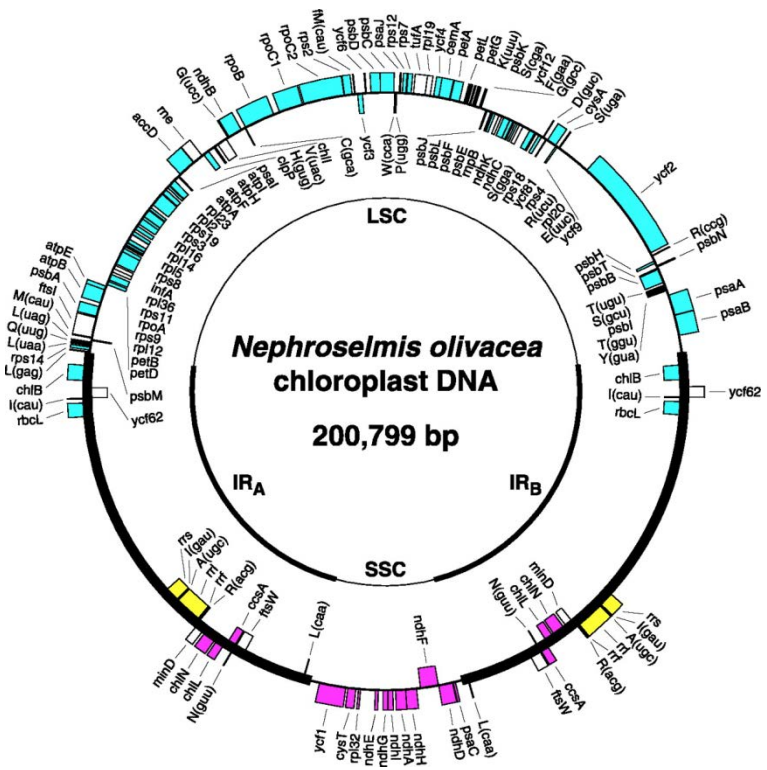
- Hybridization
- Neoteny
- Polyploidy
- Apomixis

Types of DNA data used

- Chloroplast DNA sequence data
  - Uniparentally inherited,
    - Maternal, not inherited in pollen
  - More conserved, evolves more slowly
    - Higher level relationships
    - Groupings of mulga
- Microsatellite DNA sequences
  - Biparentally inherited,
    - Genes recombine in progeny
  - Fast evolving
    - Population level relationships
    - Groupings within mulga

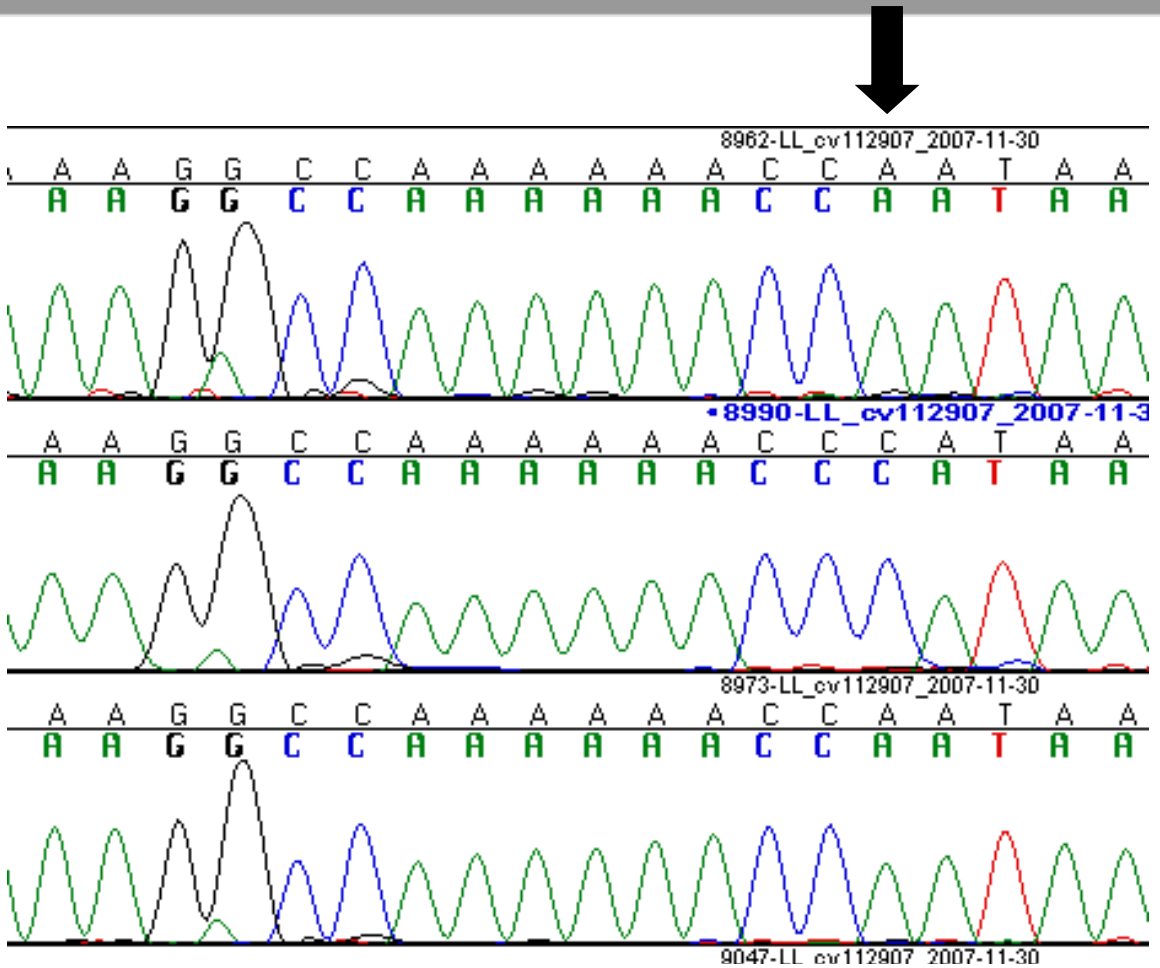


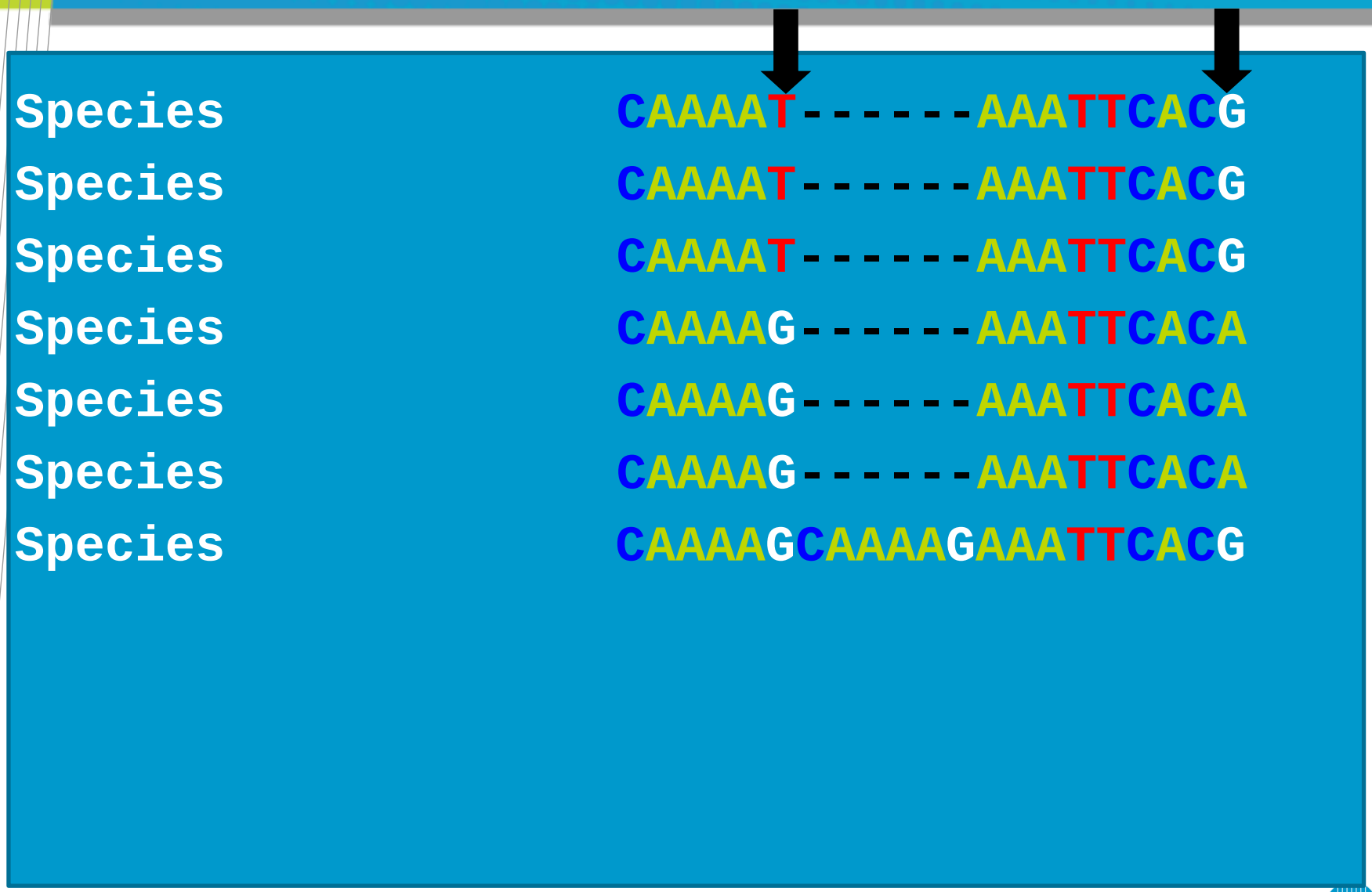
Chloroplast DNA

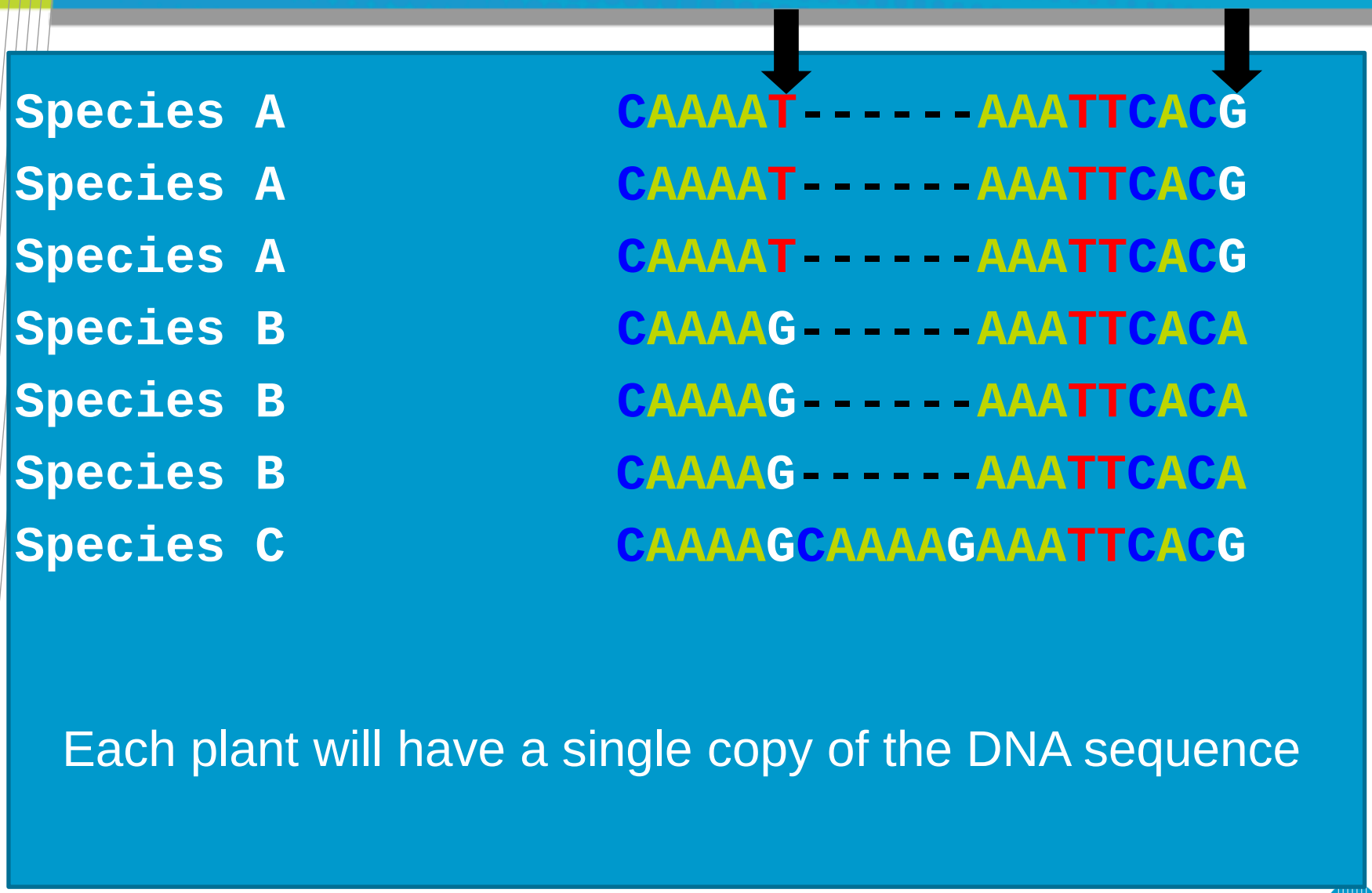


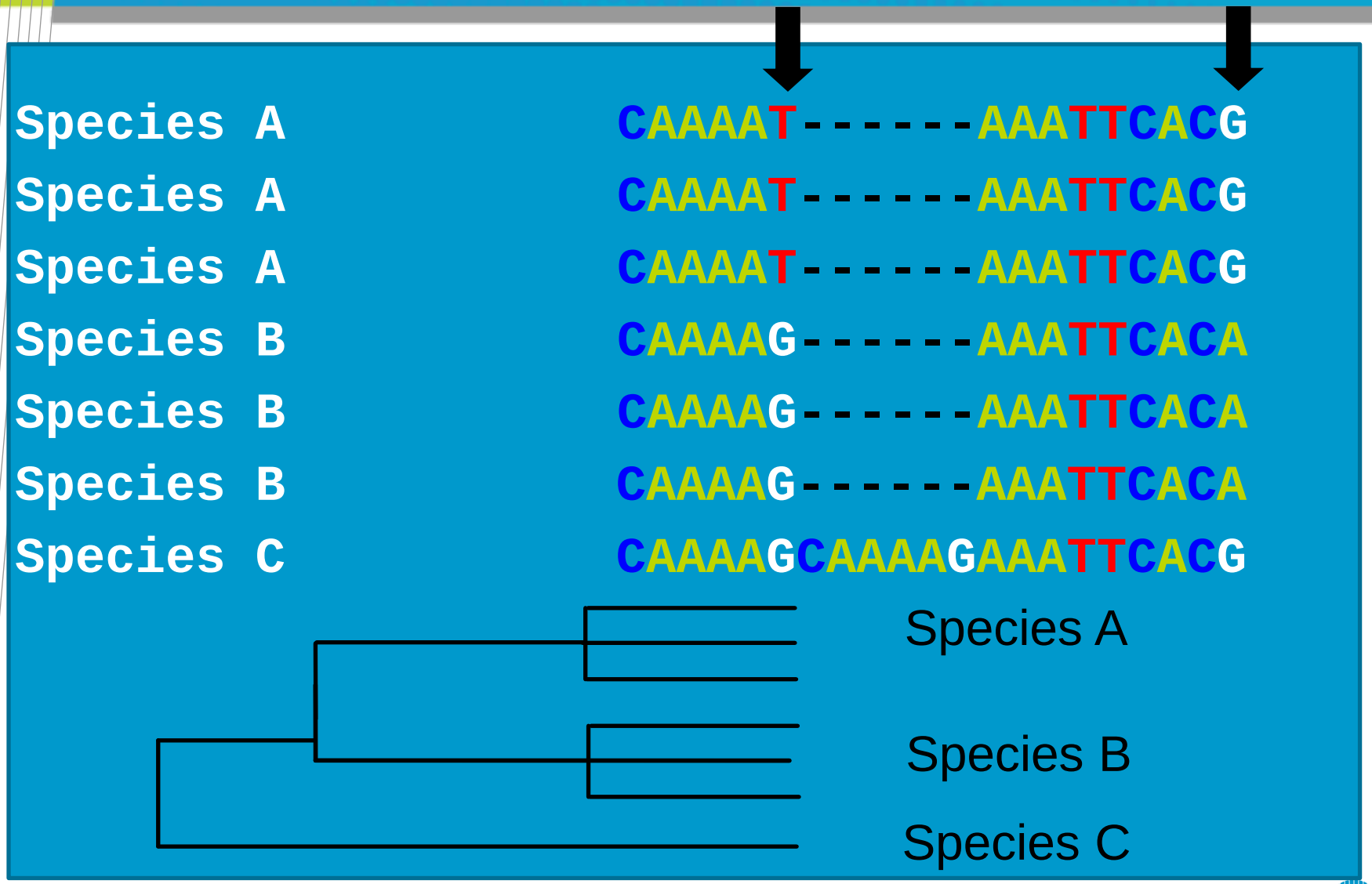
- Photosynthetic Genes
- Sequenced 4 locations
- 4,000 bp for 80 representative plants
- Goal: To determine the larger scale relationships of Mulga and close relatives

Chloroplast DNA




Species CAAAAT------AAATTCACG
Species CAAAAT------AAATTCACG
Species CAAAAT------AAATTCACG
Species CAAAAG------AAATTCACA
Species CAAAAG------AAATTCACA
Species CAAAAG------AAATTCACA
Species CAAAAGCAAAAGAAATTCACG

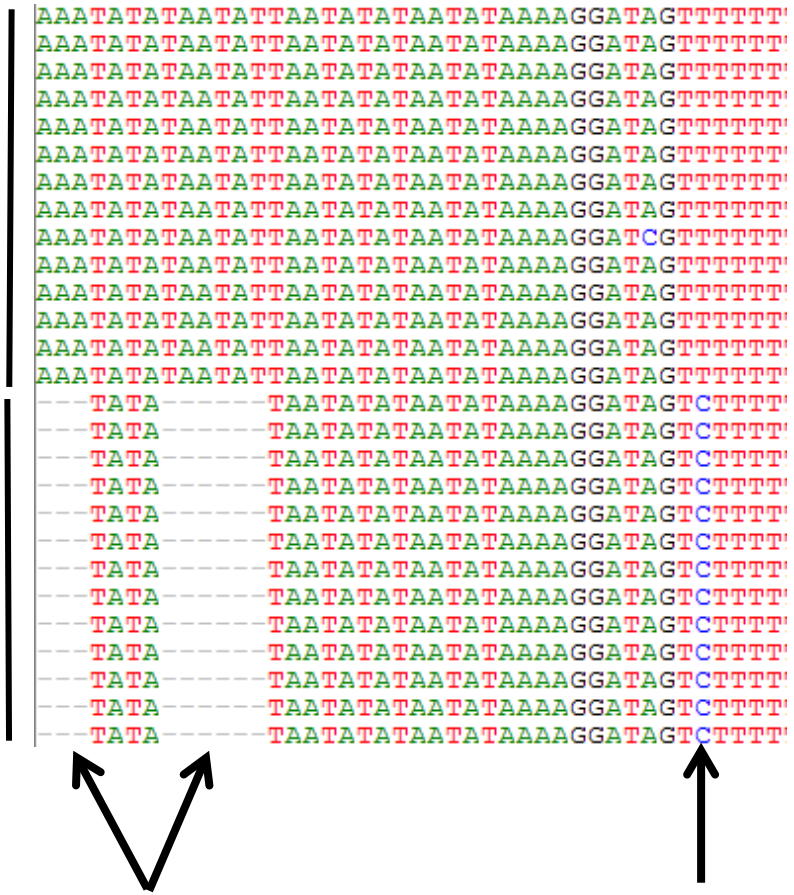

Species A CAAAAT------AAATTCACG
Species A CAAAAT------AAATTCACG
Species A CAAAAT------AAATTCACG
Species B CAAAAG------AAATTCACA
Species B CAAAAG------AAATTCACA
Species B CAAAAG------AAATTCACA
Species C CAAAAGCAAAAGAAATTCACG
Each plant will have a single copy of the DNA sequence


Species A CAAAAT------AAATTCACG
Species A CAAAAT------AAATTCACG
Species A CAAAAT------AAATTCACG
Species B CAAAAG------AAATTCACA
Species B CAAAAG------AAATTCACA
Species B CAAAAG------AAATTCACA
Species C CAAAAGCAAAAGAAATTCACG
Species A
Species B
Species C

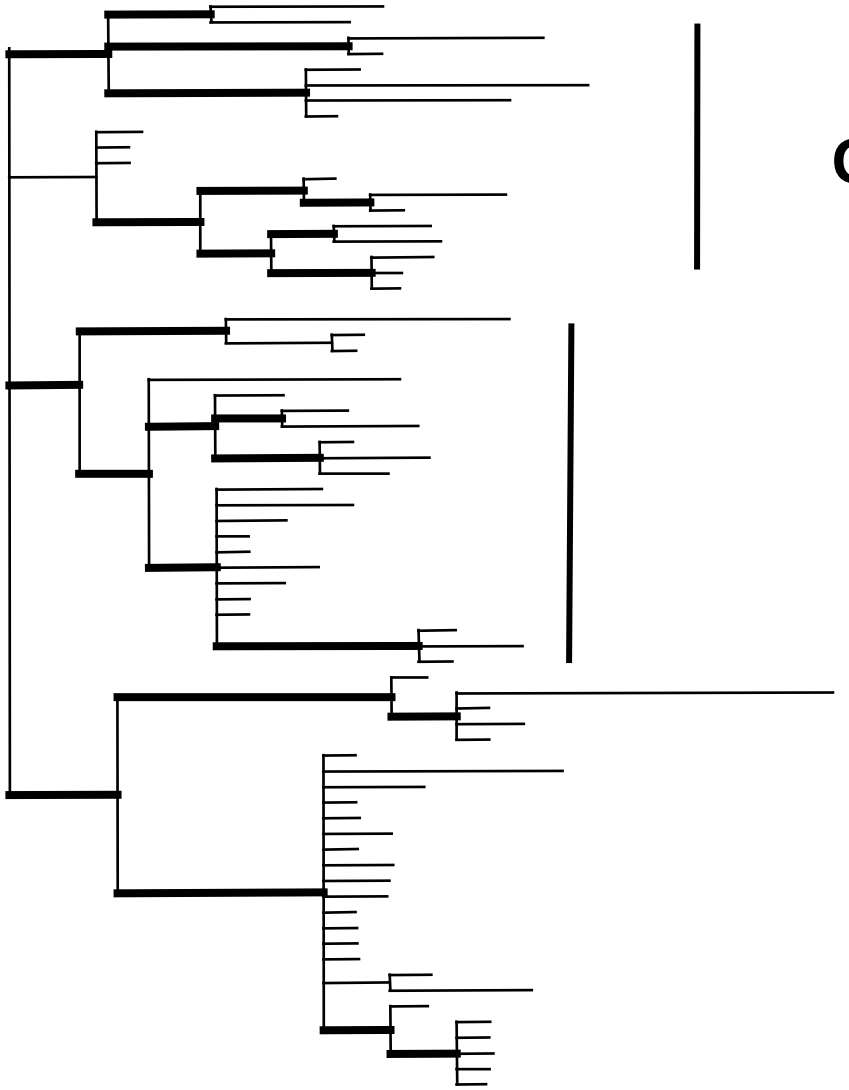
Chloroplast results

Blue Alliance

Grey-Green Alliance



Chloroplast results

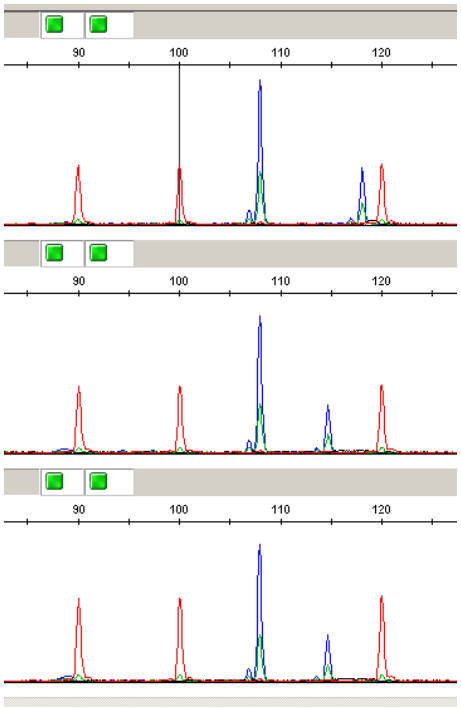


Green Alliance

Blue Alliance

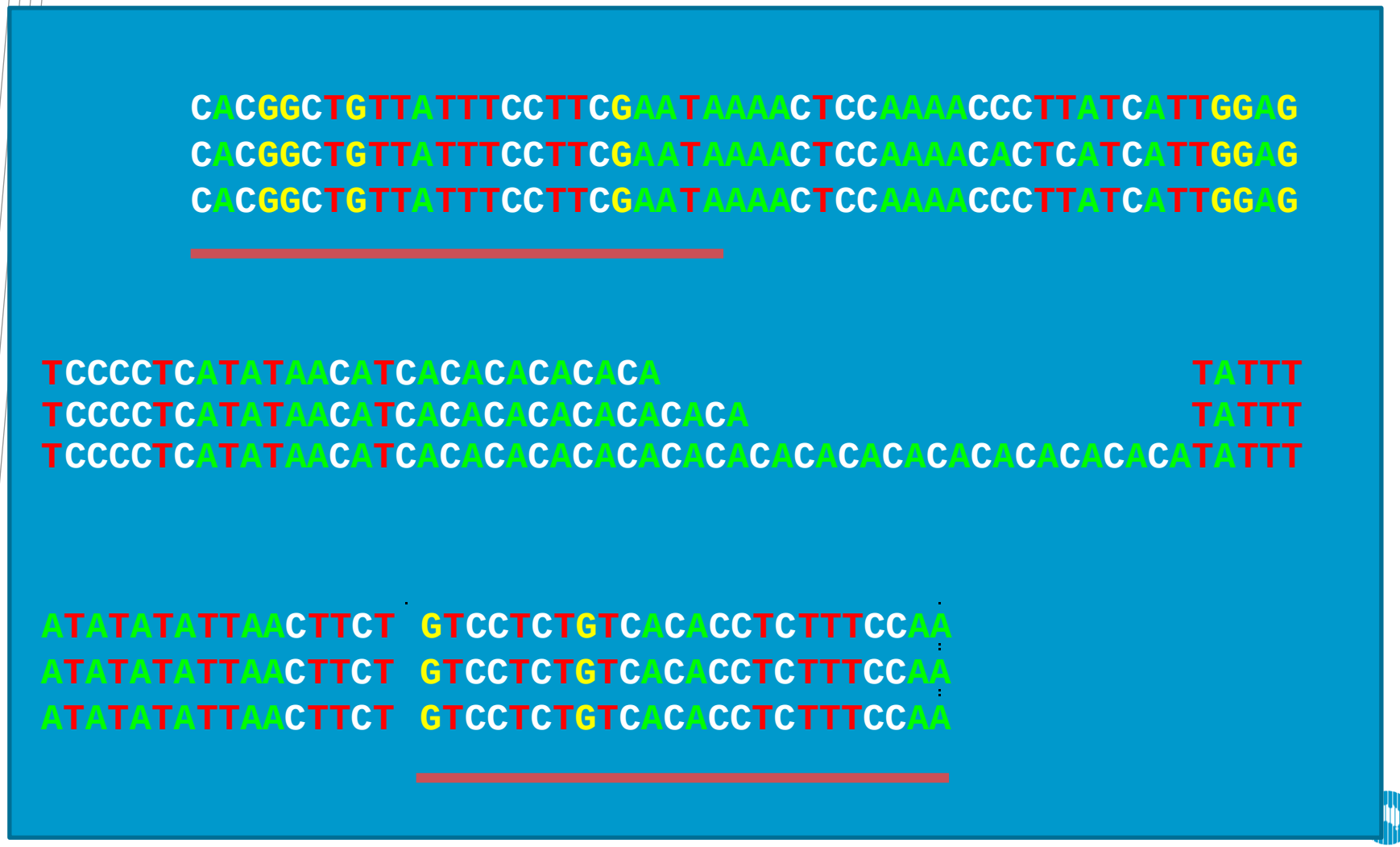
Grey-Green Alliance

Microsatellite DNA



- Non-protein coding regions of the nuclear DNA
- Usually species specific, need to be generated for each project
- Goal: To determine the finer scale relationships of Mulga and close relatives

Microsatellite DNA

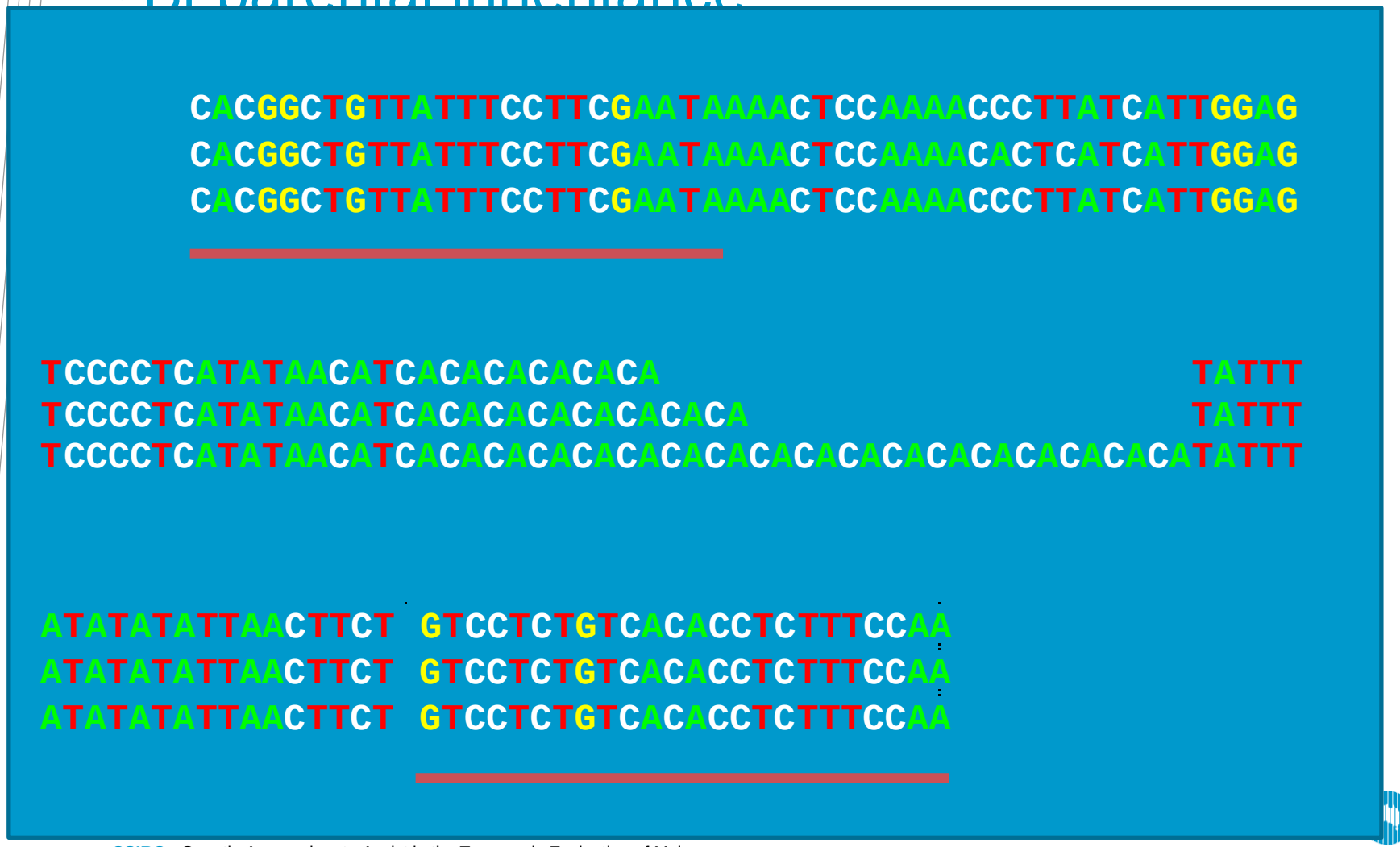


Microsatellite DNA

- Bi-parental inheritance
- An individual can be heterozygous
  - > 1 copy of the repeat
  - Different repeats (alleles) a single individual
  - Polyploids may have several alleles---
- Ability to trace individual alleles to parents
  - Within an individual
  - Within a group of plants
    - Species, morphotype

Microsatellite DNA

- Bi-parental inheritance



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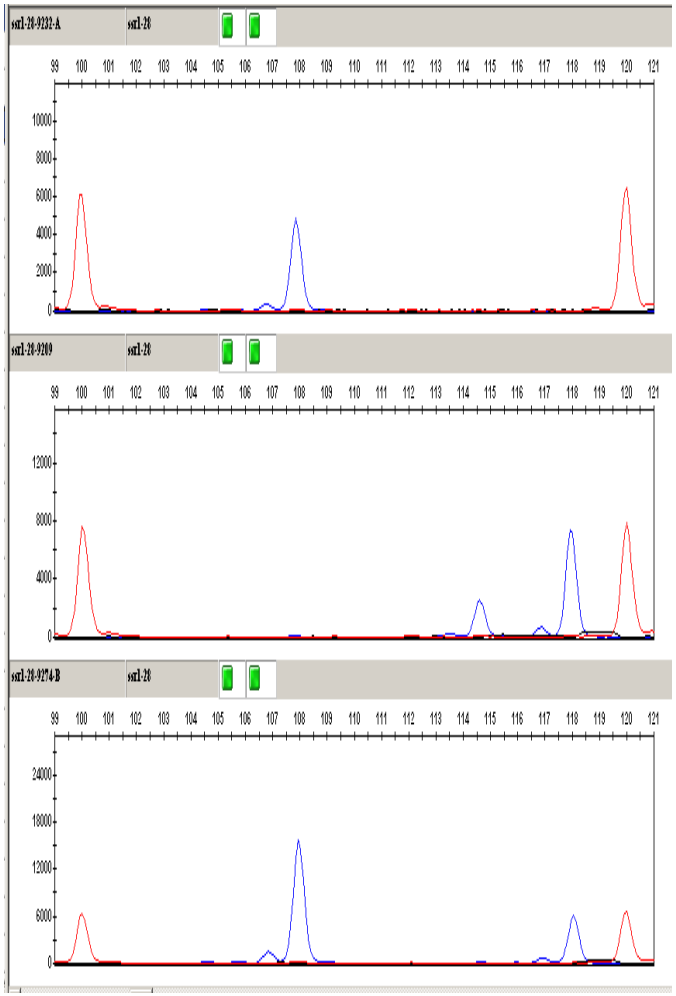
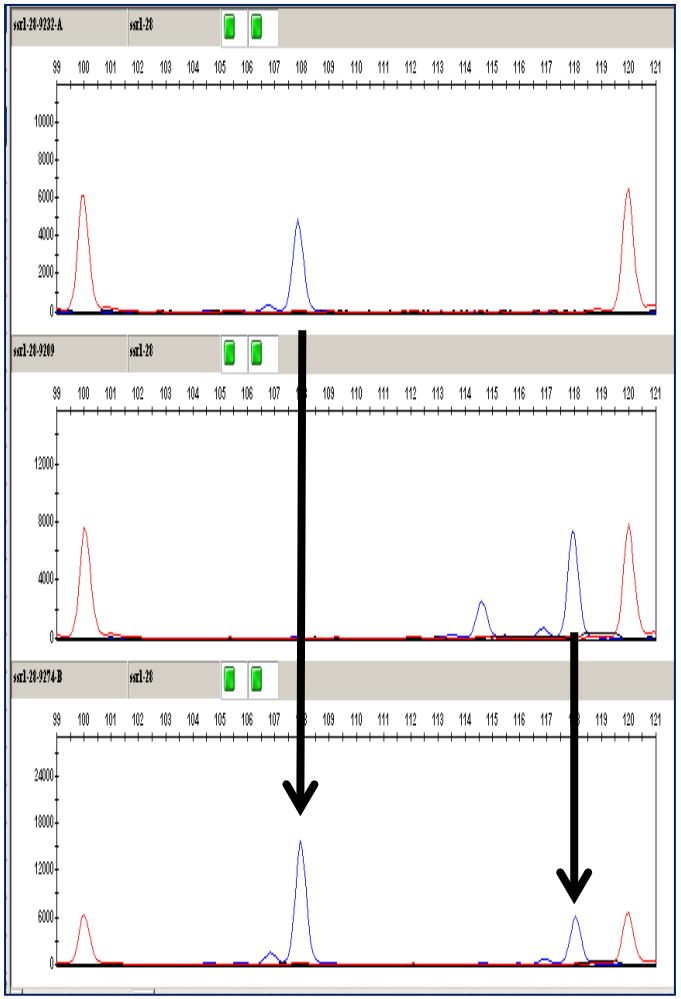


Parent 1

Parent 2

Progeny

Microsatellite DNA



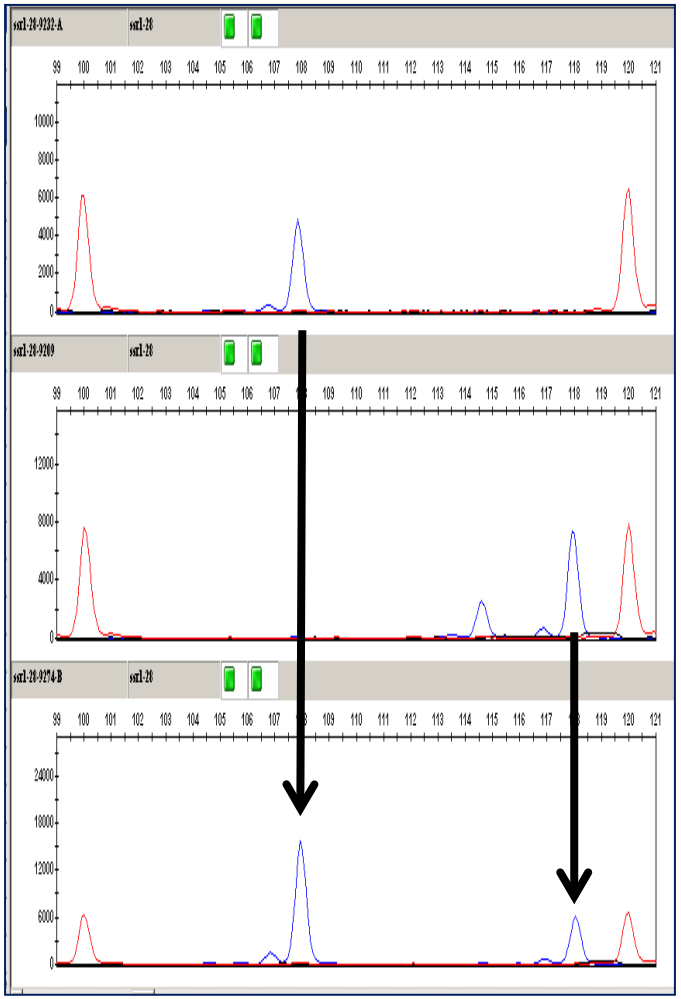
Parent 1

Parent 2

Progeny

Comparative DNA

Microsatellite DNA



Chloroplast DNA

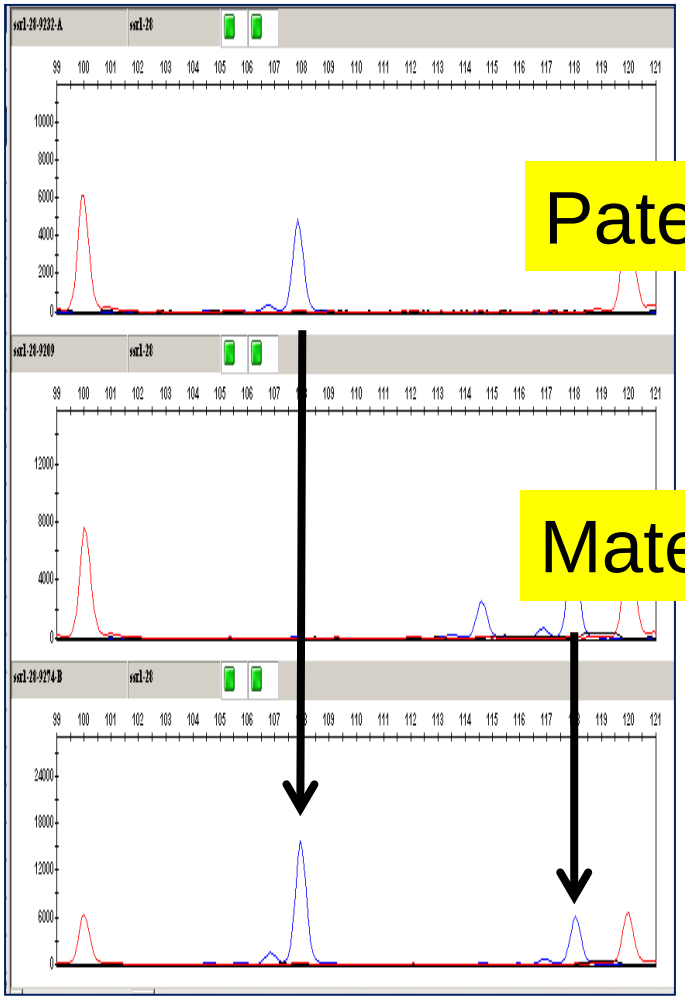
CAAAAT------AAATTCACG

CAAAAG------AAATTCACA

CAAAAG------AAATTCACA

Comparative DNA

Microsatellite DNA



Chloroplast DNA

CAAAAT------AAATTCACG

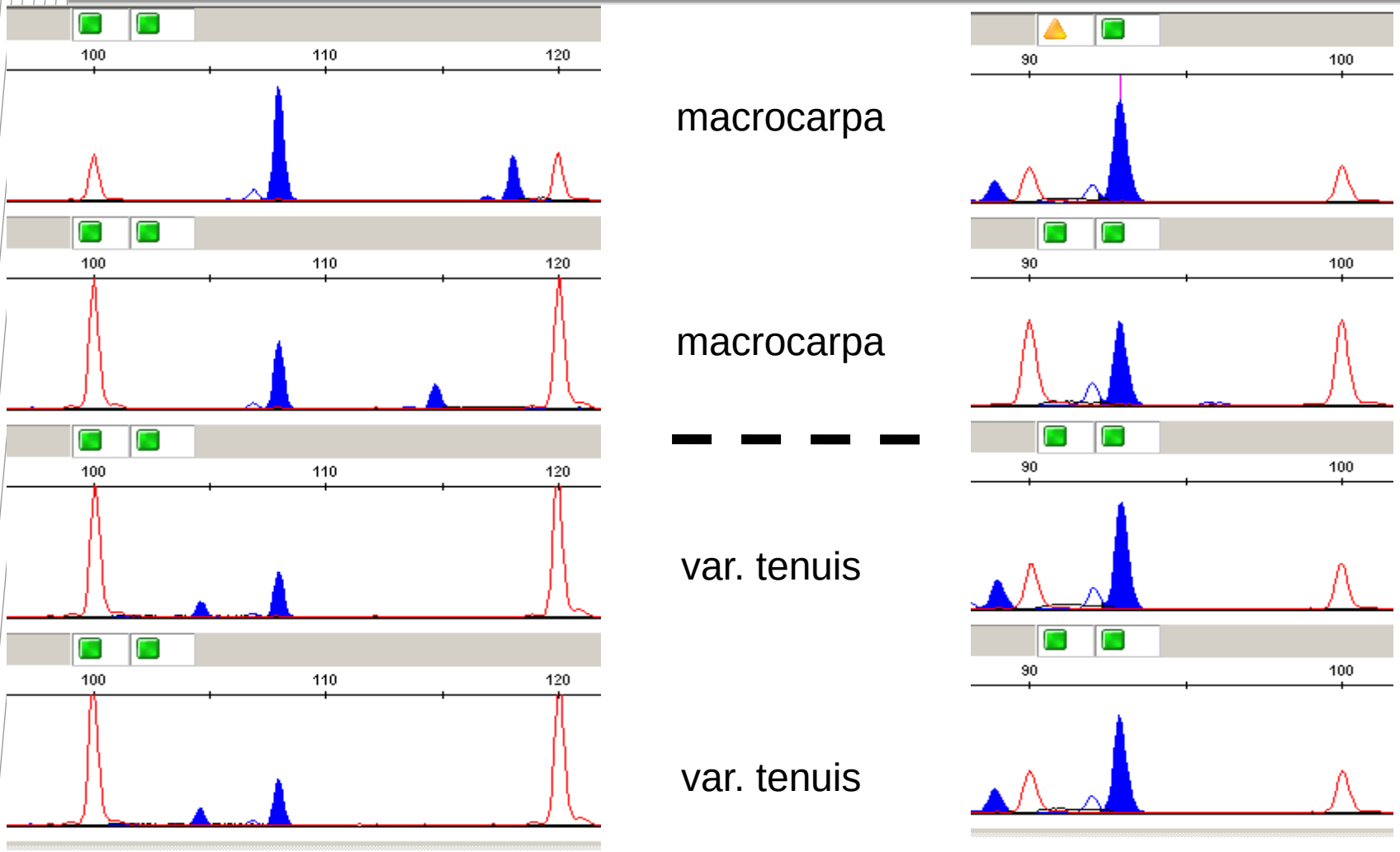
Paternal parent

CAAAAG------AAATTCACA

Maternal parent

CAAAAG------AAATTCACA

Within the Green Alliance

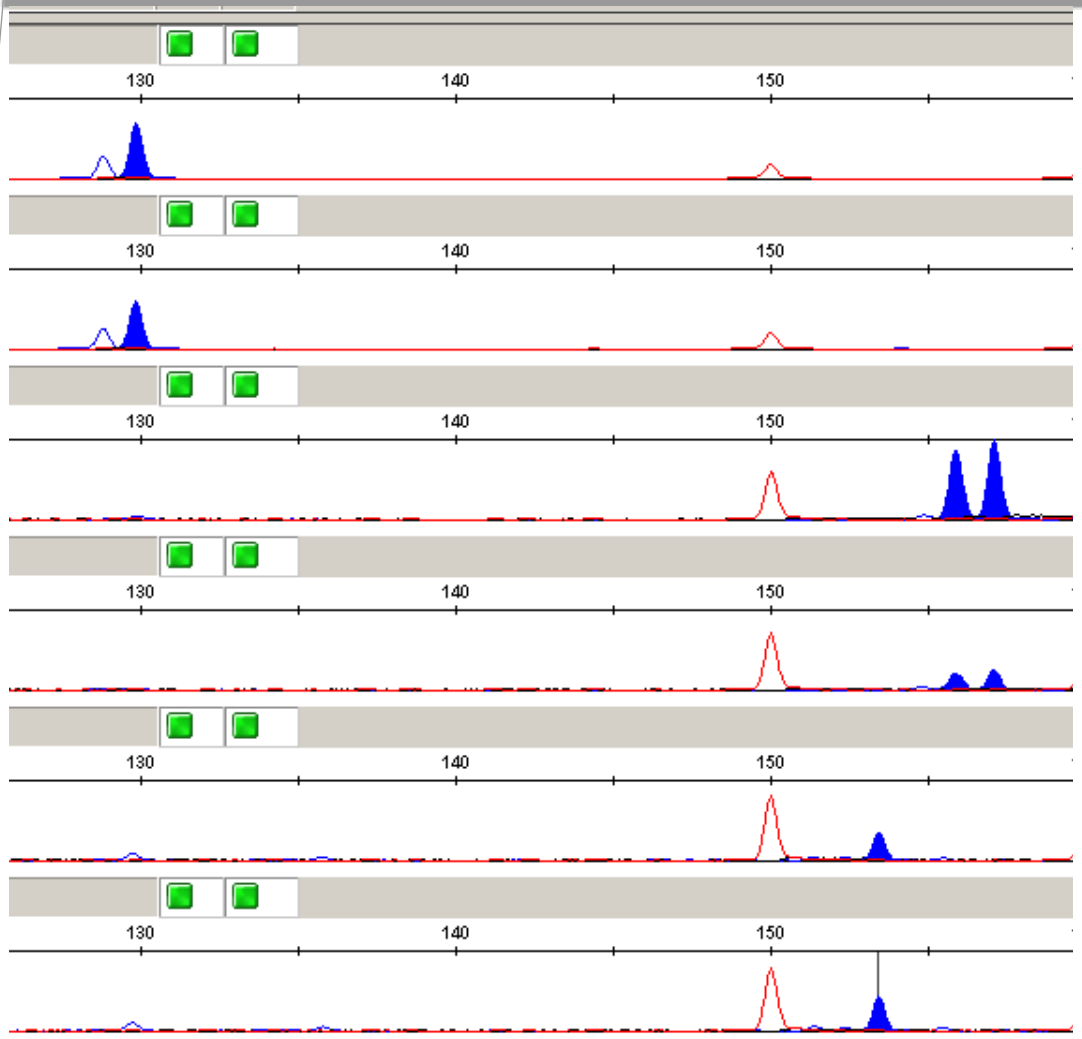


Locus 1-28

Locus 12-2

No variation within var. tenuis in this population

Patterns specific to alliances



Green Alliance

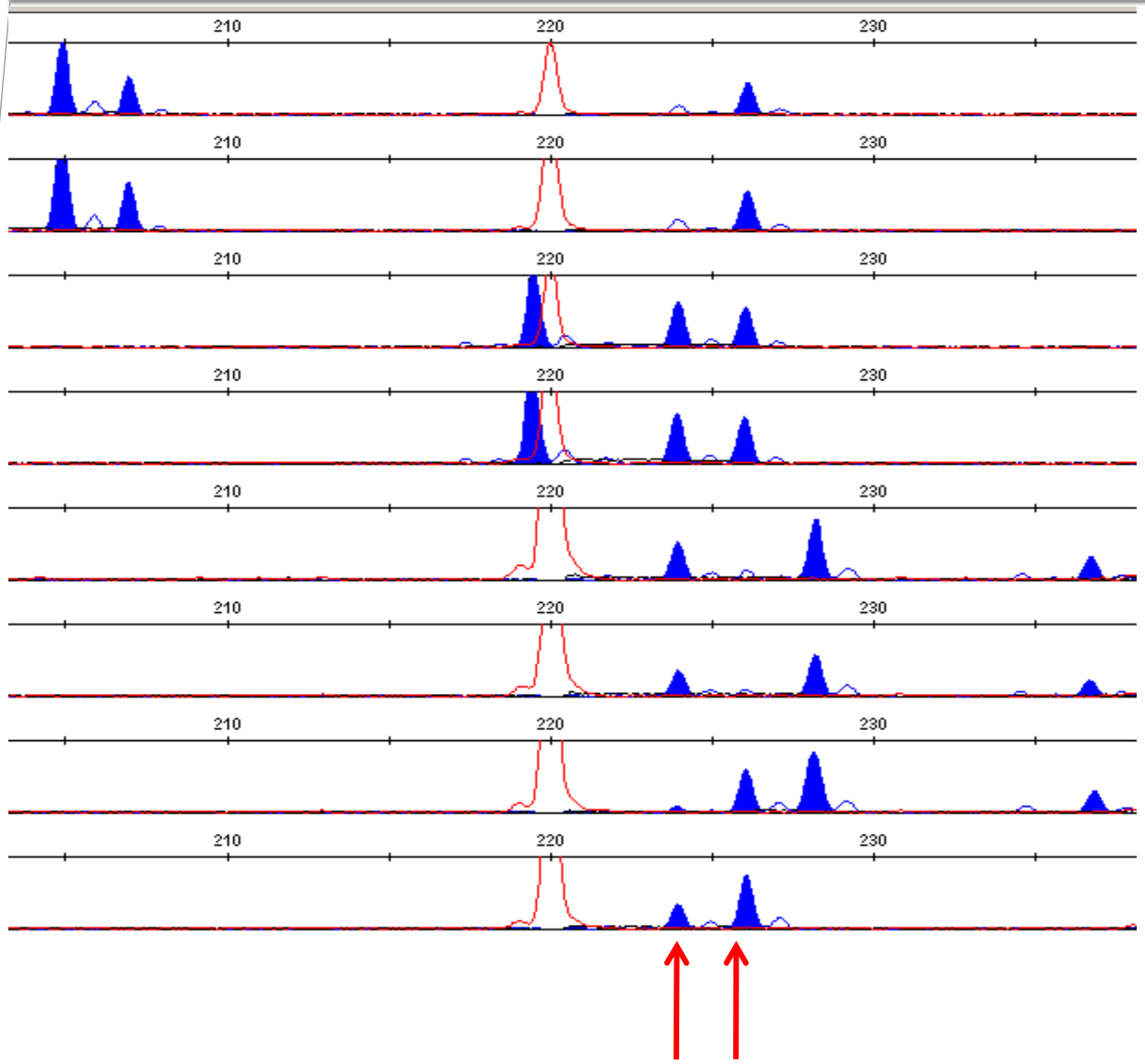
Blue Alliance

Grey-Green Alliance

Difficult to find markers specific to an Alliance

CSIRO

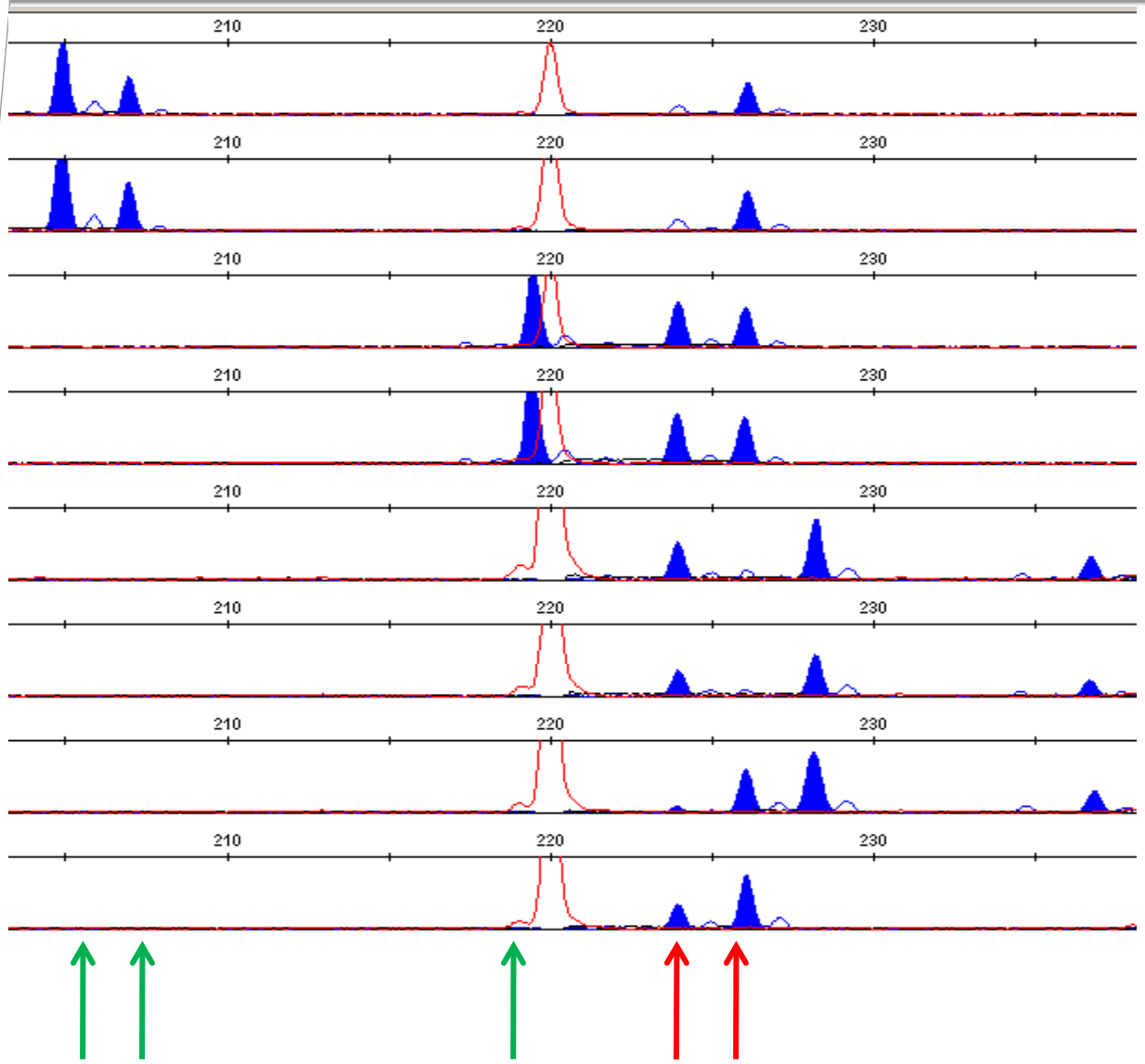
Shared and specific markers



Green Alliance

Blue Alliance

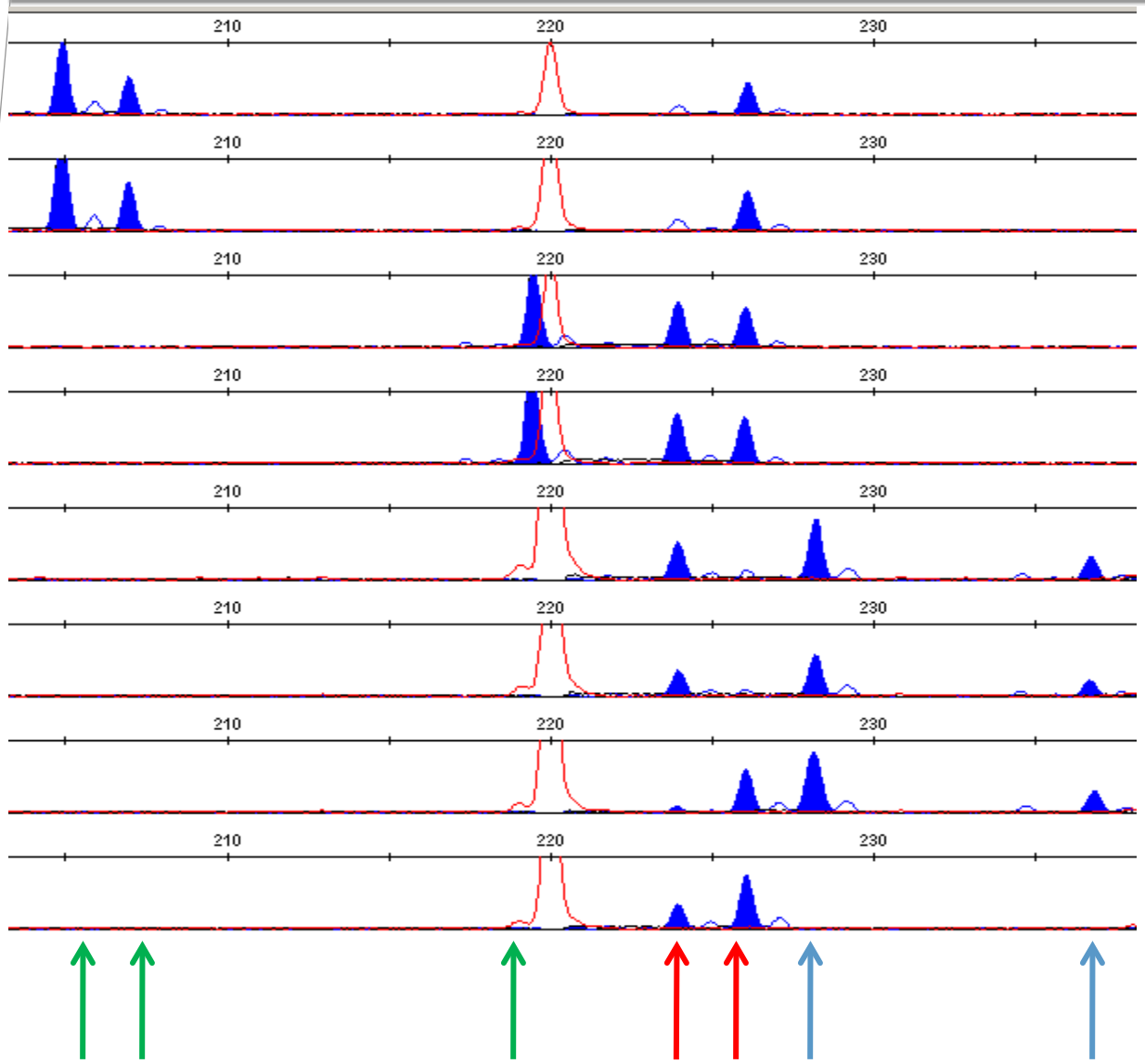
Shared and specific markers



Green Alliance

Blue Alliance

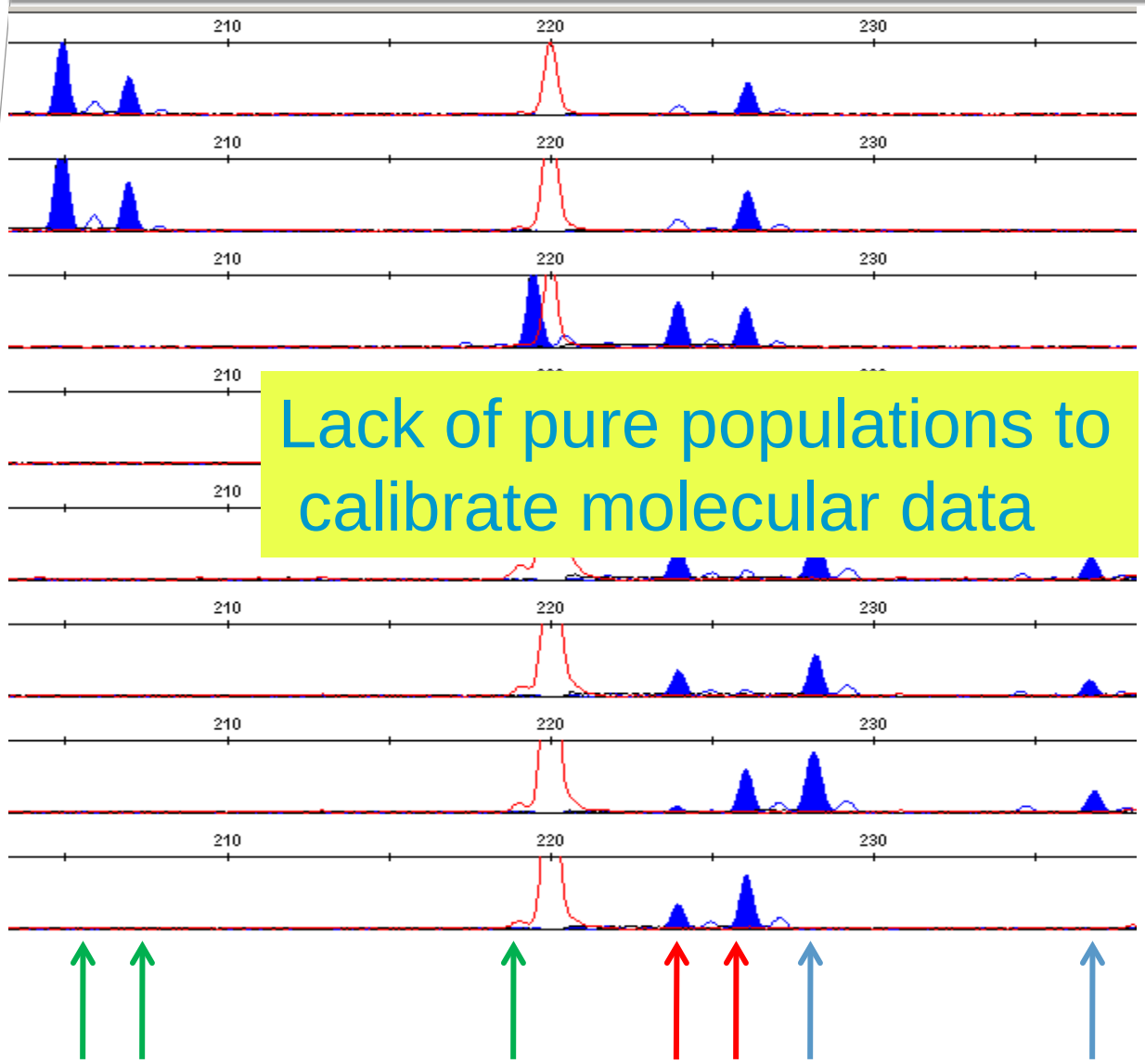
Shared and specific markers



Green Alliance

Blue Alliance

Shared and specific markers

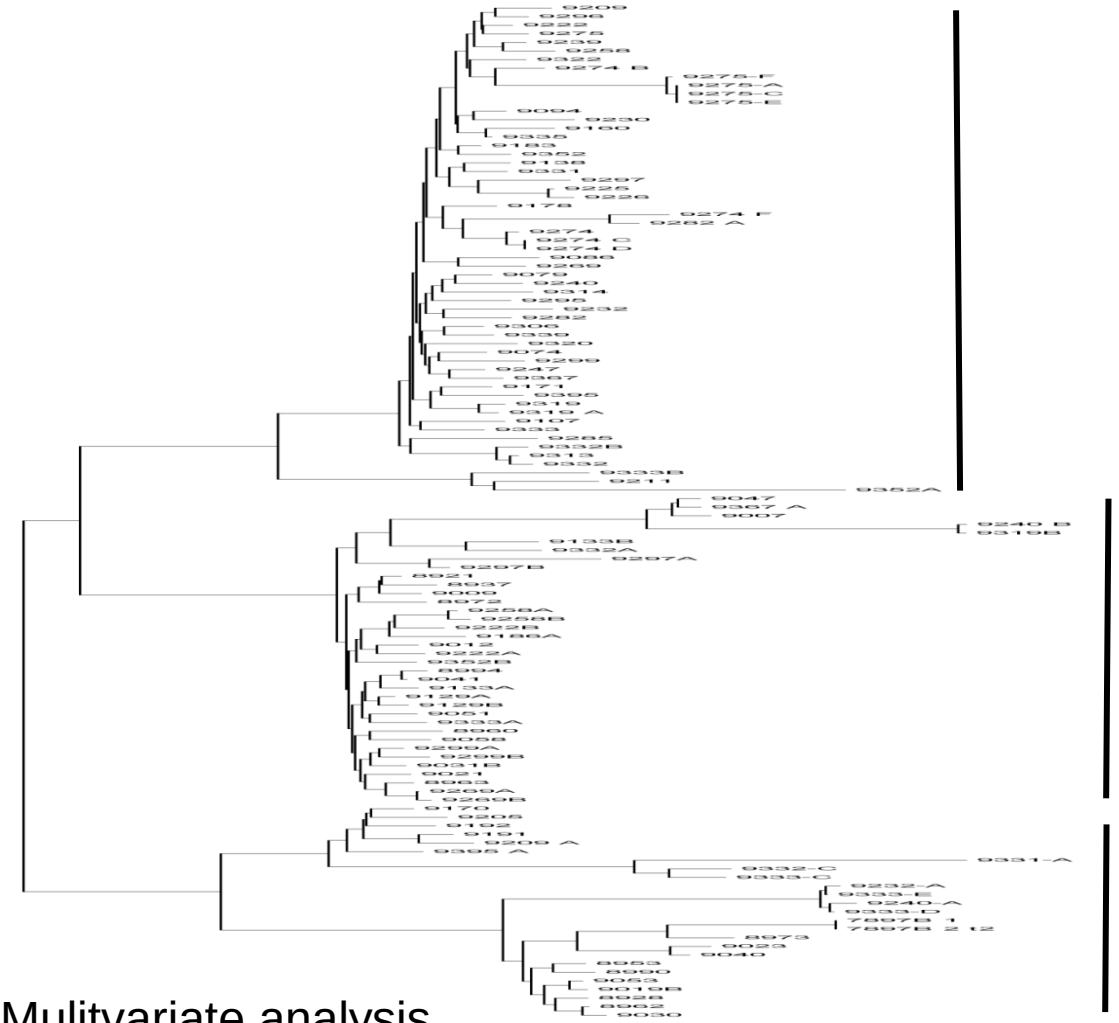


Lack of pure populations to calibrate molecular data

Green Alliance

Blue Alliance

Microsatellite results



Mulitvariate analysis

Green Alliance

Grey-Green Alliance

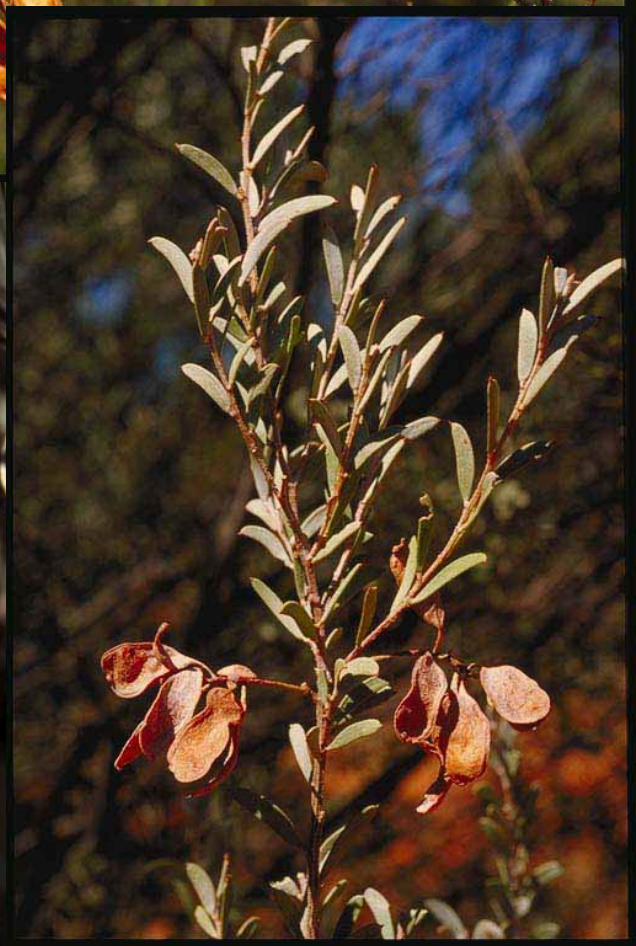
Blue Alliance

Comparison of data types

- Each shows three groupings
  - Green Alliance
  - Grey-GreenAlliance
  - Blue Alliance

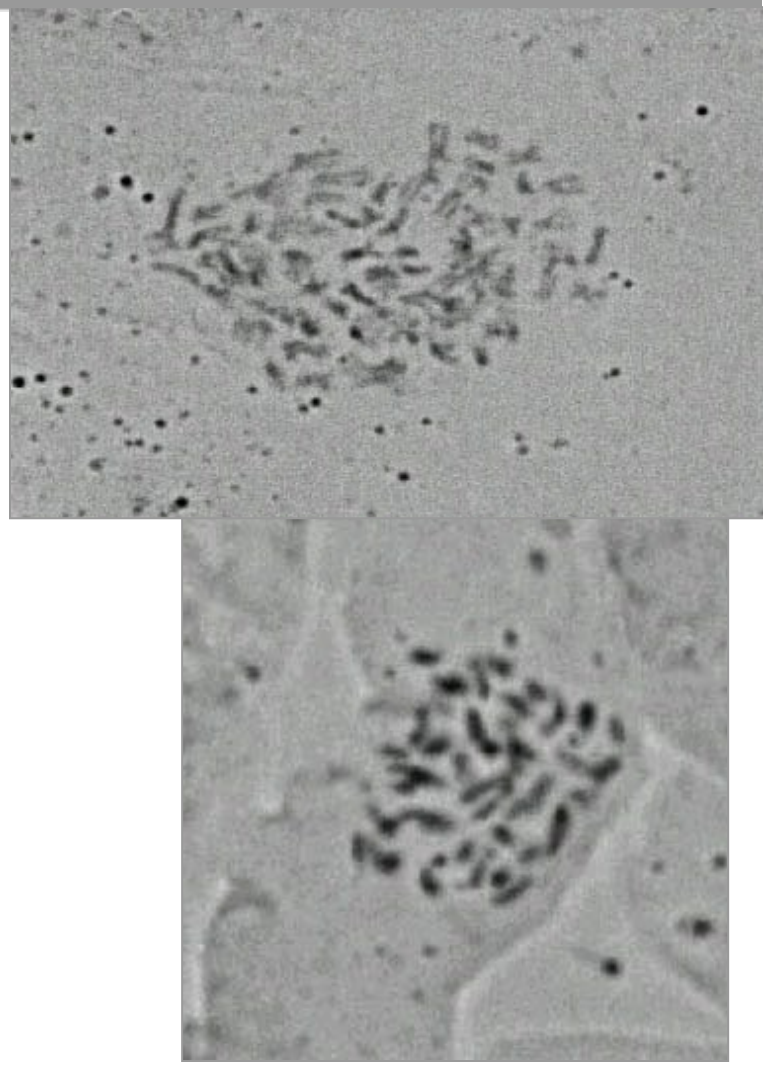
- Three groups found in morphology

Genetic Factors

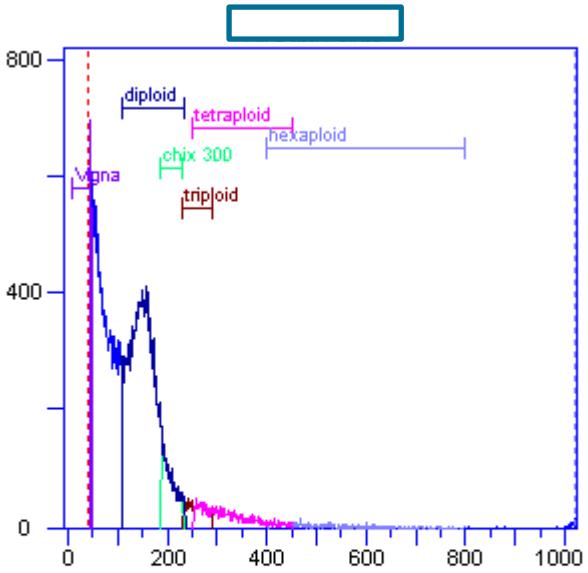


Polyploidy

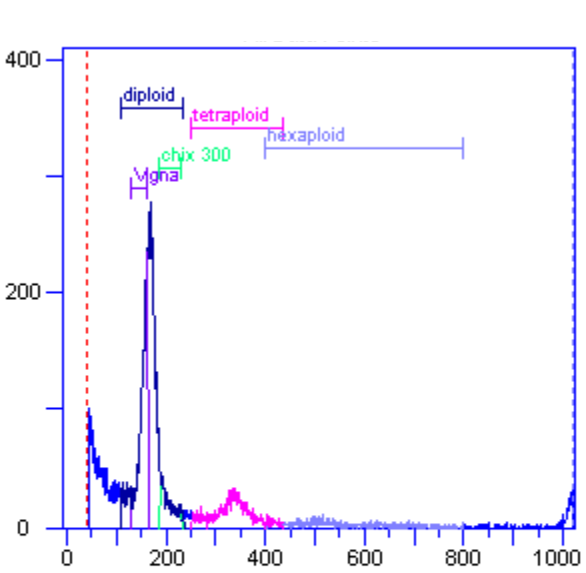
- Measure of the sets of chromosomes
- Mostly tetraploid (2n = 52)
- Some hexaploids(2n = 78)
- Diploid Mulga close relatives



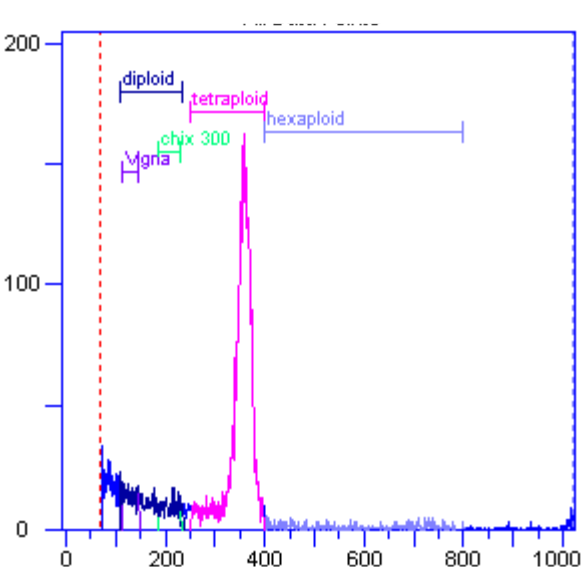
Outgroups: Diploid



Acacia thoma

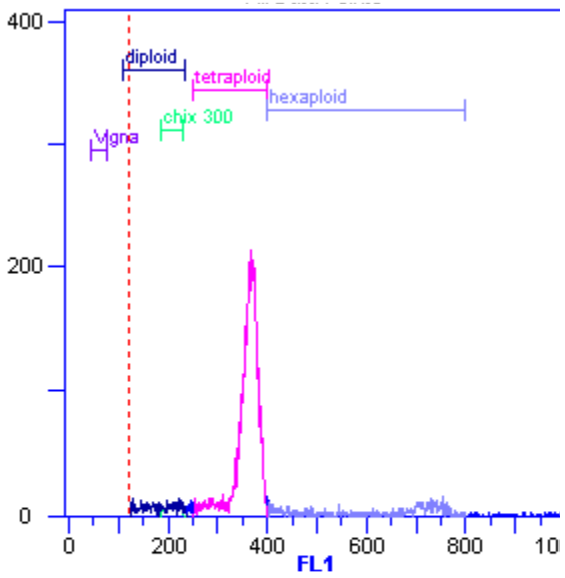


A. ramulosa

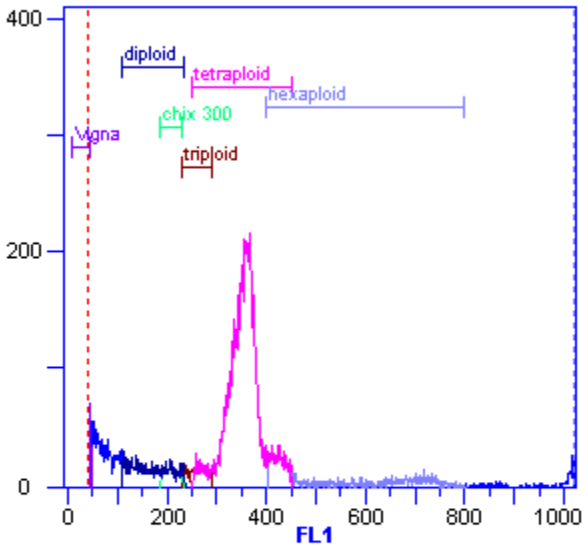


A. ramulosa X *A. aneura*

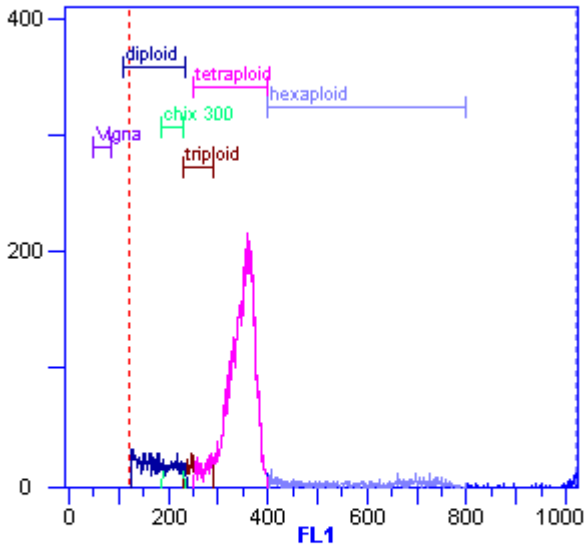
Tetraploids



paraneura

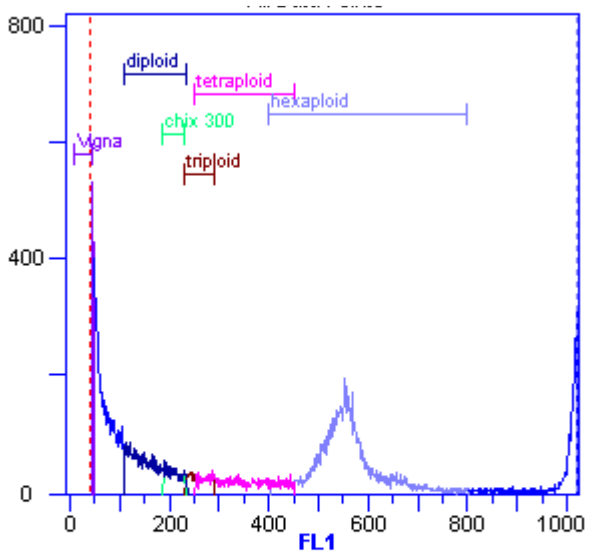


tenuis

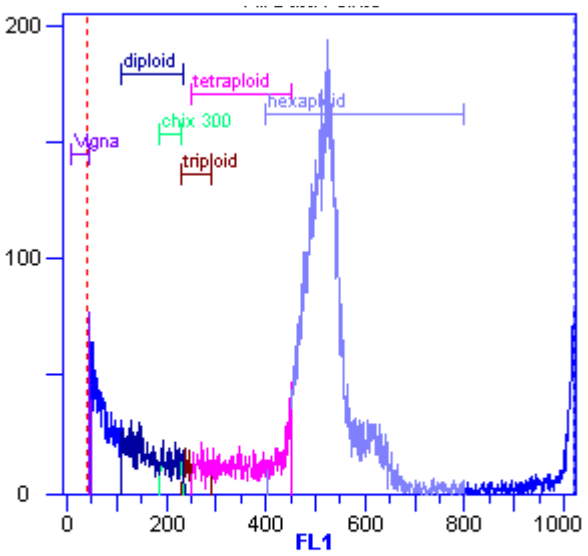


tenuis

Hexaploids



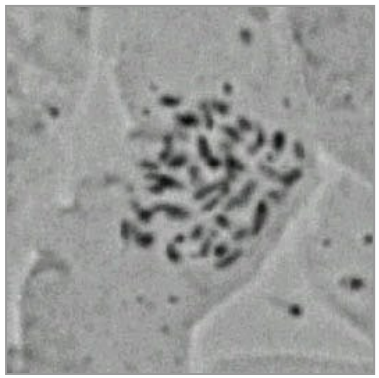
paraneura



var. tenuis flat

Polyploidy

- Does ploidy level correlate with morphotype?
- Ploidy level differences act as barrier to sexual reproduction
  - Triploid infertility
- Polyploids of multiple origin may show greater variation

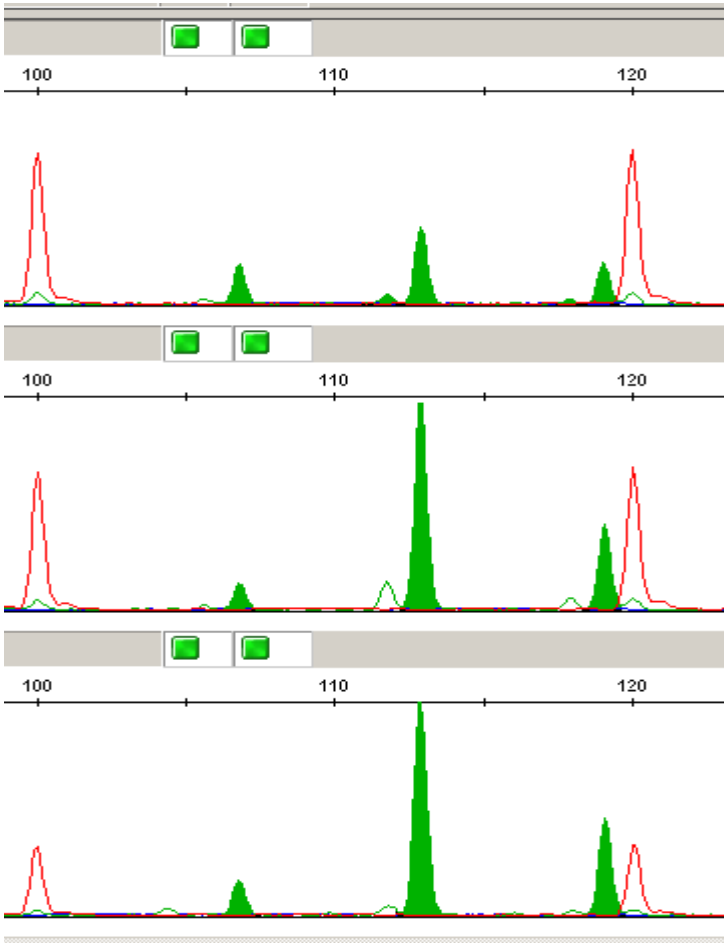


Apomixis

- Asexual reproduction through seed
- Apomicts commonly polyploid
- Method to bridge gap between ploidy levels because sexual reproduction is not needed
- **Variants are maintained**

Polyembryony

Apomictic parent and progeny

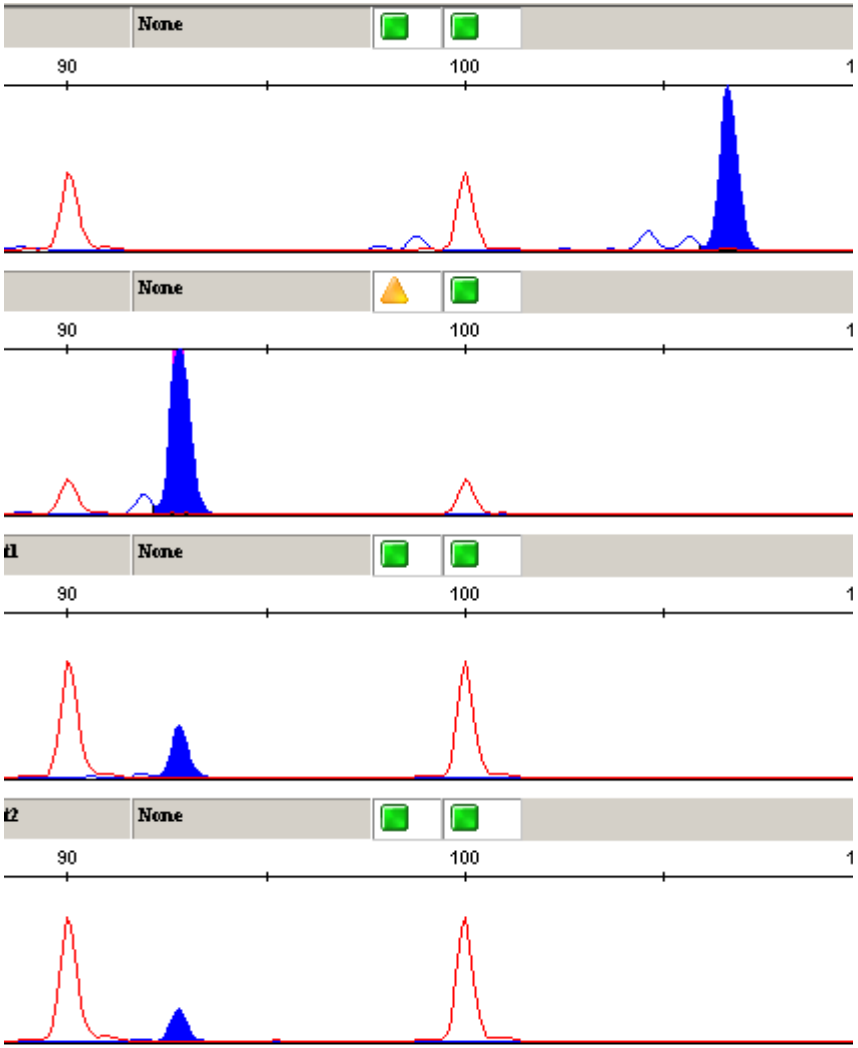


Locus A15

Progeny genotype is identical to mother at all loci

CSIRO

Population variation



Locus 12-2

Same population

parent plant

twin progeny

twin progeny

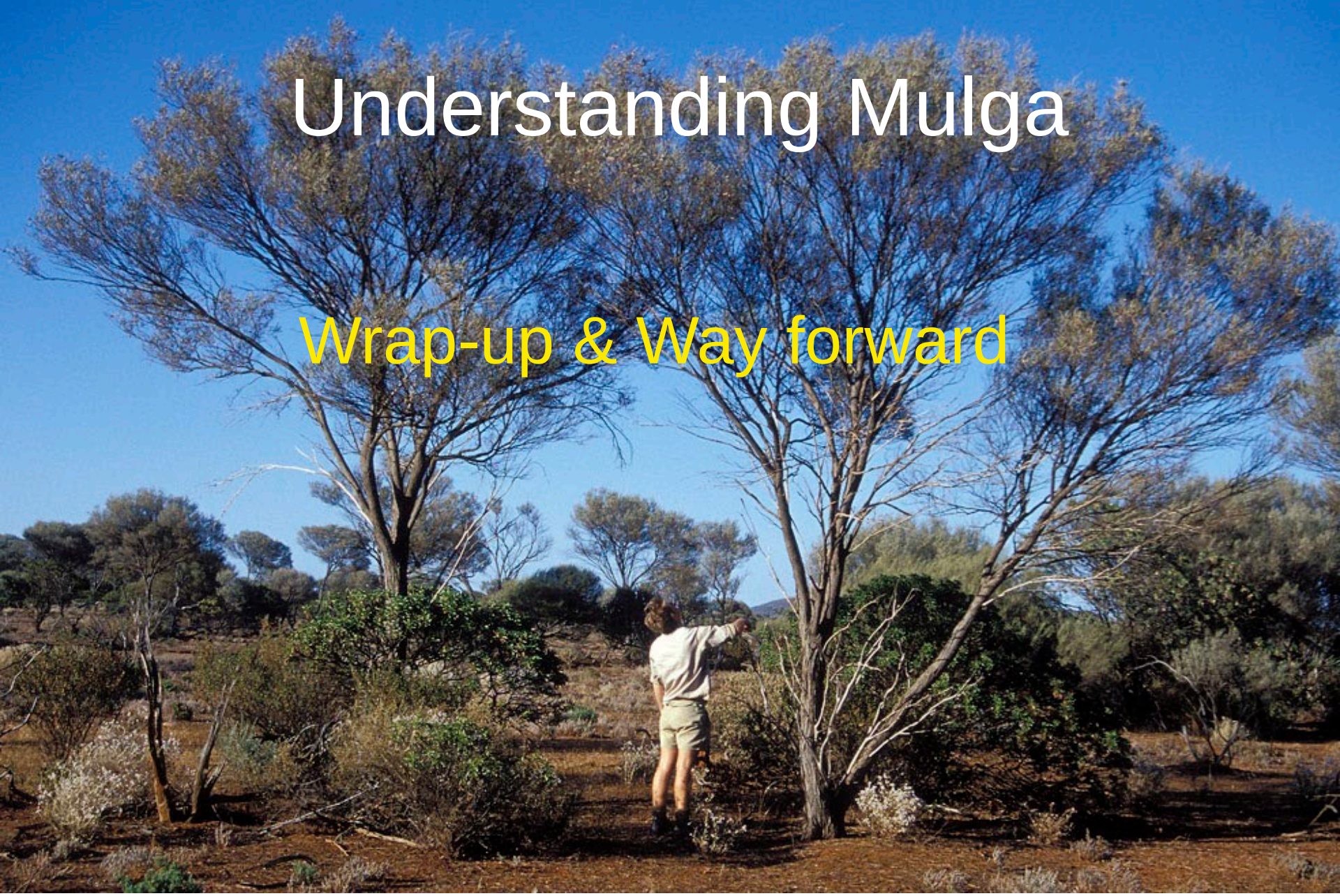
Summary

- Genetic evidence (chloroplast and microsatellite DNA) for three alliances
- Correlates with taxonomic data

- Are the factors acting equally in the complexes?
  - Hybridization
  - Polyploidy
  - Neoteny
  - Apomixis

Understanding Mulga

Wrap-up & Way forward



bhpbilliton RIO TINTO FMG ACT NOW FOR THE FUTURE Department of Environment and Conservation

Our environment, our future

Understanding Mulga project outcomes

DIRECT OUTCOMES

1. Produce comprehensive taxonomic review of Mulga
2. Determine genetic factors responsible for creating & maintaining Mulga diversity
3. Construct key (electronic) to identify Mulga types
4. Develop website for *Understanding Mulga* project
5. Assess conservation status of Mulga types in W.A.
6. Produce publications
    - scientific (taxonomic & genetic)
    - *Mulga Manual* (hardcopy & web)

DERIVED OUTCOMES

1. Provide information for effective management, conservation & utilisation of Mulga,
2. Provide basis for understanding Mulga over its geographic range
3. Provide a framework for undertaking & interpreting applied ecological studies involving Mulga.

Understanding Mulga project outcomes

DIRECT OUTCOMES

1. Produce comprehensive taxonomic review of Mulga
2. Determine genetic factors responsible for creating & maintaining

The *Mulga Manual*

- **Description & illustrations of different Mulga types**
- **Description of Mulga biology, ecology, distribution**
- **For use by resource managers, researchers, regulators & educators**
- **Produced also as a simplified version for non-specialist users**

1. Provide information for effective management, conservation & utilisation of Mulga,
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1. Taxonomic review

Major achievements

- Conceptual framework established (Green, Blue, Grey-green alliances)
- **22** W.A. taxa recognized following character analysis (vegetative & pod)
- Taxon descriptions commenced

To do

- Consolidate/verify existing taxa recognized; ?recognition of additional taxa; develop methods to reliably recognize hybrids
  - Undertake field & herbarium study
  - Complete anatomy & ontogeny studies
- Undertake study of inflorescence characters
- Complete taxon descriptions & illustrations

Constraints

- Geographic (long distances)
- Phenological (flowers/pods rainfall dependent)
- Seedlings (need time to mature; develop germination techniques)

2. Genetic studies

Major achievements

- Development of microsatellite libraries
- Identification of chloroplast genes
- Genetic recognition of the three Mulga alliances
- Commenced study of Mulga ploidy level

To do

- Optimize microsatellite methodology
- Generate genetic data on entire range of Mulga samples
- Test taxonomic hypotheses using genetic data
- Determine genetic factors responsible for variation in Mulga

Constraints

- Geographic & phenological as per taxonomic study
- Lack of pure populations to calibrate molecular data
- Movement of principal investigator from Iowa to Canberra
- Acquisition of flow cytometer (to progress ploidy level study)

3. Electronic key

Major achievements

- Draft key commenced

To do

- Optimize characters employed in key
- Code all Mulga taxa
- Assemble & test key

Constraints

- Effectiveness of key is dependent upon taxonomic discrimination of Mulga types
- Ability to effectively key hybrids & minor variants

4. Mulga websites

Major achievements

- Two websites created:
  - WorldWideWattle (DEC)
    http://www.worldwidewattle.com/infogallery/projects/mulga.php
  - University of Iowa
    http://www.biology.uiowa.edu/newccg/jmiller/mulga/php
- Partners acknowledged on both websites

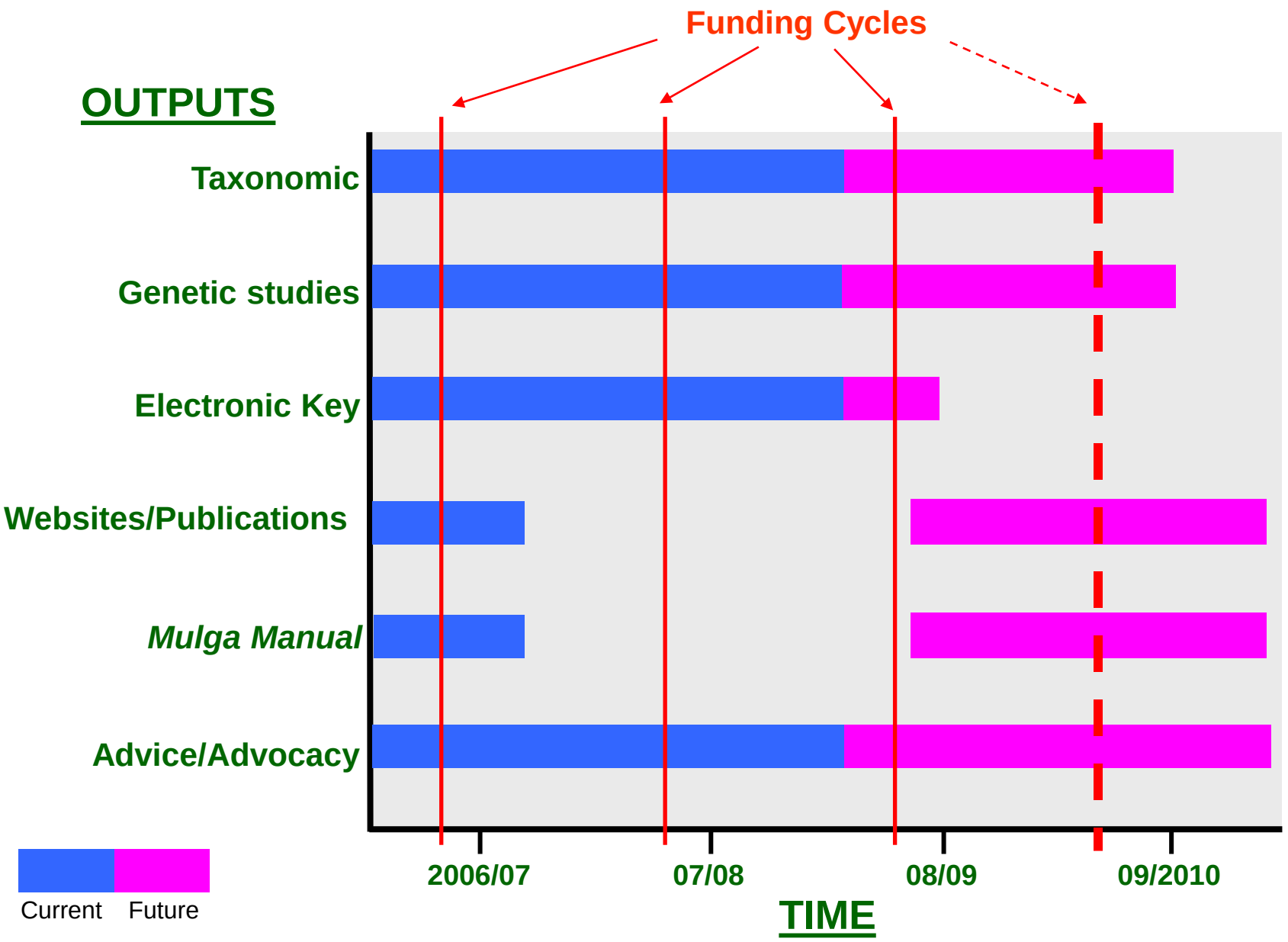
To do

- Maintain currency of WorldWideWattle website

5. & 6. Conservation status & publications

Unable to undertake activities until taxonomic & genetic outputs are complete

Progress Summary



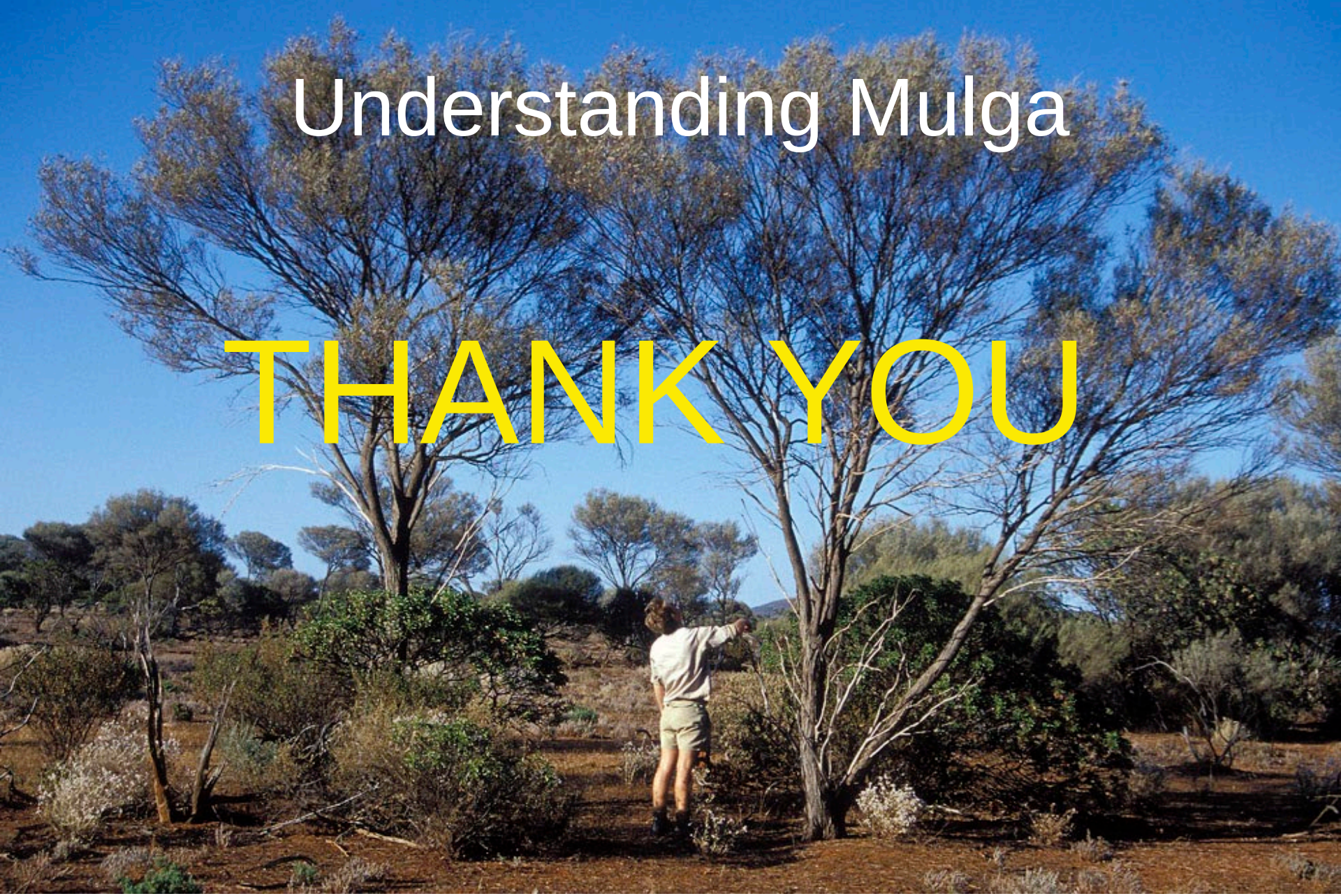
Budget 2009/2010

TO BE CONFIRMED

$140K in salary & on-costs

$40K in material/equipment costs

Understanding Mulga



THANK YOU

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