Pre-translocation preparation – using a model incorporating a genotype collection method

David Taylor

Overview

- Documenting conservation collections – why we need a collecting method

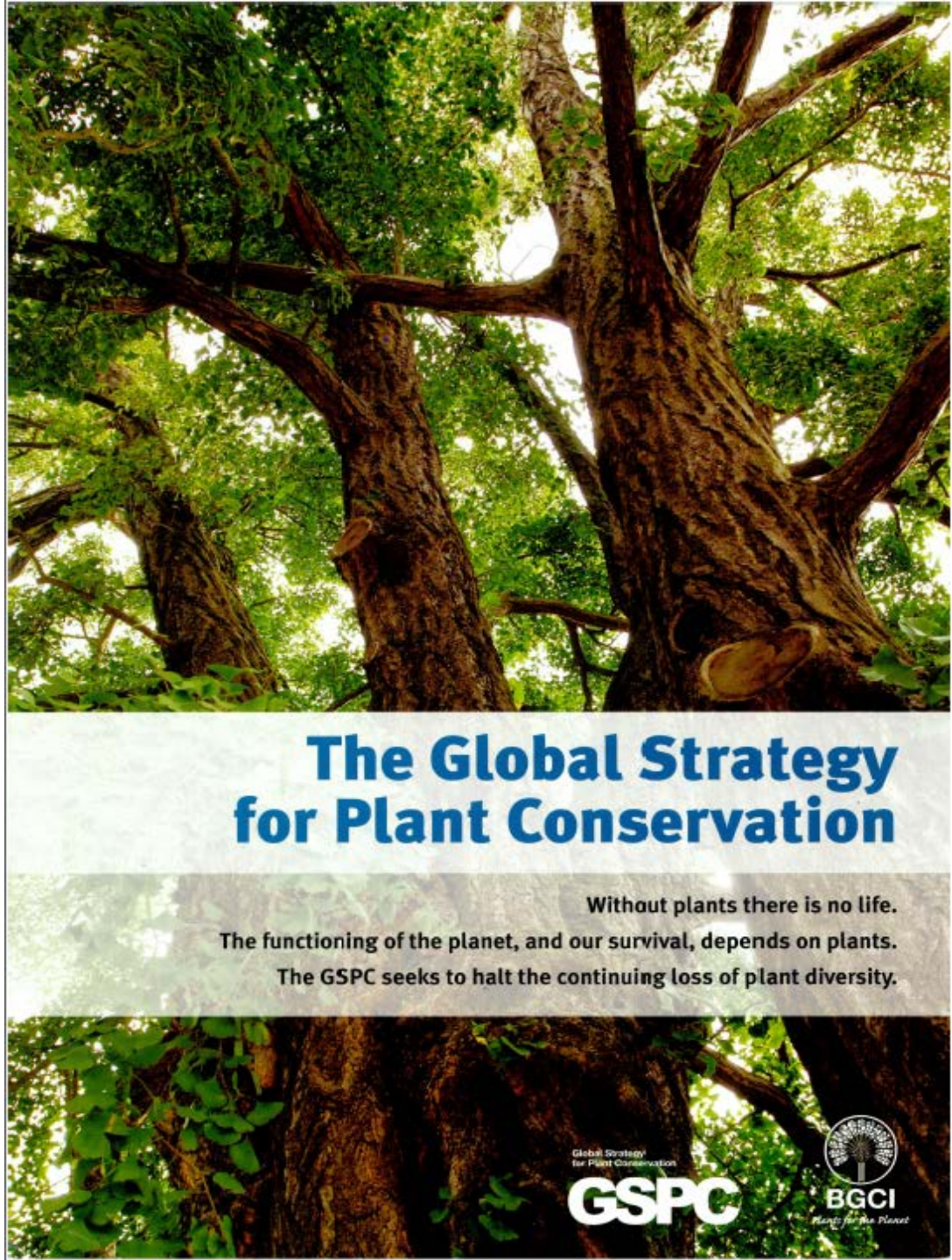
- The benefits of a genotype collecting method

- A genotype collection method in action - Pomaderris

Documenting conservation collections
why we need a collecting method

- **The global strategy for Plant conservation**
- Relevant Plant target:

  Target 8 'At least 75% of Threatened Plant Species in ex-situ collections, preferably in the country of origin, **and at least 20% available for recovery and restoration programs…**



Why is genetic diversity important for ex-situ conservation collections and for delivering successful translocations?

- Genetic diversity in ex situ collections is good because therein may lie the alleles or traits for e.g. pathogen resistance, stress tolerance or other traits that may benefit the endangered species if exposed to new threatening processes
- In the common situation where such data are not available, conservation scientists and agencies tend to advise 20-30 individual plants should be sampled per population

Documenting conservation collections
why we need a collecting method

- When collecting propagation material (either using seed and non-seed seed methods) for translocation, a critical and often overlooked factor is the ability to **link ex-situ individuals to parentage or wild origin** which identifies the population source and potential genetic diversity available.

A genotype collecting method has many benefits

- It can be used to help address the important connection between wild and translocated plants

- Incorporated into a checklist it can provide a user friendly tool for a diverse range of users

The key objectives of a genotype collection method is to provide a user friendly tool that will:

- Be able to trace source populations and have control over genotype selection for future translocations.
- Maximise the chance of a successful collection event and enable populations locations and specific plants sampled to be readily revisited (where applicable) for follow-up collecting.
- Enable each individual/team to be armed with a user-friendly guide and reference to enable efficient and effective collecting and to ensure a standard protocol for field collections is adopted.
- Be appealing and practical for a wide audience with the goal of it being adopted and used widely and as a standard, which will enable better comparative analysis across projects and easier access to key standardised information and terminology.

What does a genotype collecting method look like ?

What can it achieve?

A 3 year Environment Trust research project

Initiated by the **South East NSW Bioregion working group** partners
targeting a minimum of

5 threatened *Pomaderris* species

The Pomaderris Project is assisted by the New South Wales Government through its Environmental Trust.

So prior to determining the collection method
It is essential to:

1. Identify the Objective(s)!

2. Establish Protocol(s)!

Pomaderris

- Objectives
  - Secure seed & vegetative material (long term storage, and potential restoration)
  - Investigate biological traits (seed germination & cytology)
  - Produce plants for translocation projects
- Protocols
  - Standard protocol for field collections
    - Based on typical herbarium and field collecting guidelines
  - Prescriptive protocol for *Pomaderris* project
    - Complements & supports the Standard Protocol
    - Ensures we can revisit populations and specific plants for follow up collecting.
    - Ensures all partners are armed with a user friendly guide and reference to enable consistent, efficient and effective collecting methods.

Standard protocol for field book collections

- A collection number and co collectors.
- Date.
- Each sample must have a GPS record. For this project using WGS 84 datum including seconds. This makes it easier to accurately make a return visit.
- GPS accuracy in meters.
- A basic description of the locality.
- A brief description of the size of the plant.
- The Pomaderris project number corresponding to the metal tag (when sampling from tagged plants).
- Elevation in meters.
- Aspect.
- Topography.
- Soil type/substrate if known.
- Each species locality should be represented by at least one herbarium specimen (with duplicates) and noted as such in the field note book.
- Note the abundance of the species targeted at the locality (Often best done retrospectively).
- What was collected (cuttings or seed)
- Field name of the plant

Prescriptive protocol for Pomaderris project

- Establish
  - extent of population.
- Tag
  - 5 plants with permanent tags. This should be done in numeric order and ideally be spread out amongst the sample area.
- Collect material from **30** plants
  - vegetative material (cuttings) from the 5 tagged plants. Record individual collections.
  - Collect seeds from the same 5 plants. Record individually and different to the cuttings!
  - Collect seeds from another 25 plants. 10 m minimum distance between plants.
- *Timing*
  - *2, if not 3, site visits may be required.*
  - *Collect a herbarium specimen containing flower or fruit.*
- *Note:* This prescriptive protocol integrates with the Standard protocol **for field collections.**

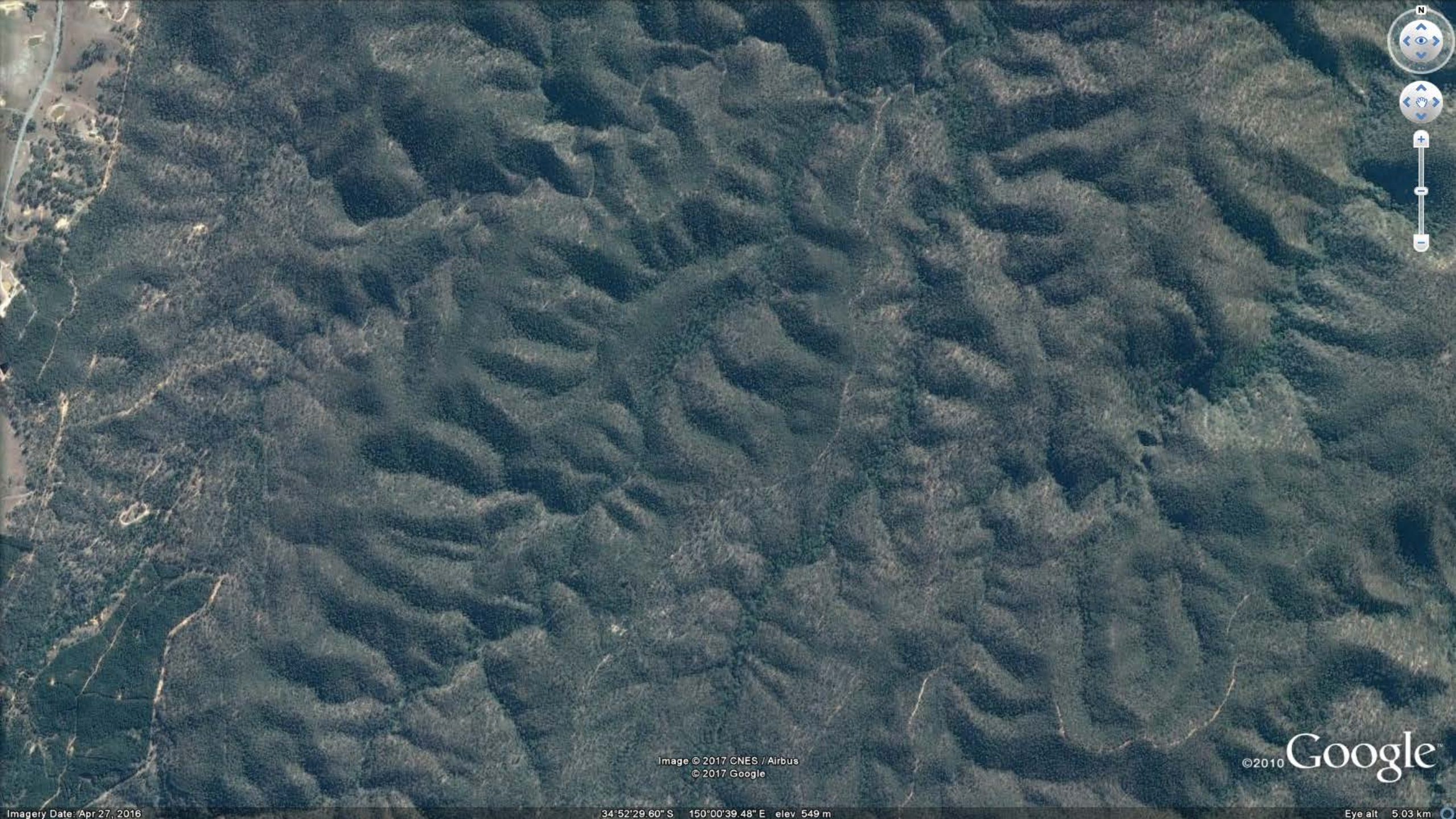


Image © 2017 CNES / Airbus
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Imagery Date: Apr 27, 2016
34°52'29.60" S 150°00'39.48" E elev 549 m
Eye alt 5.03 km

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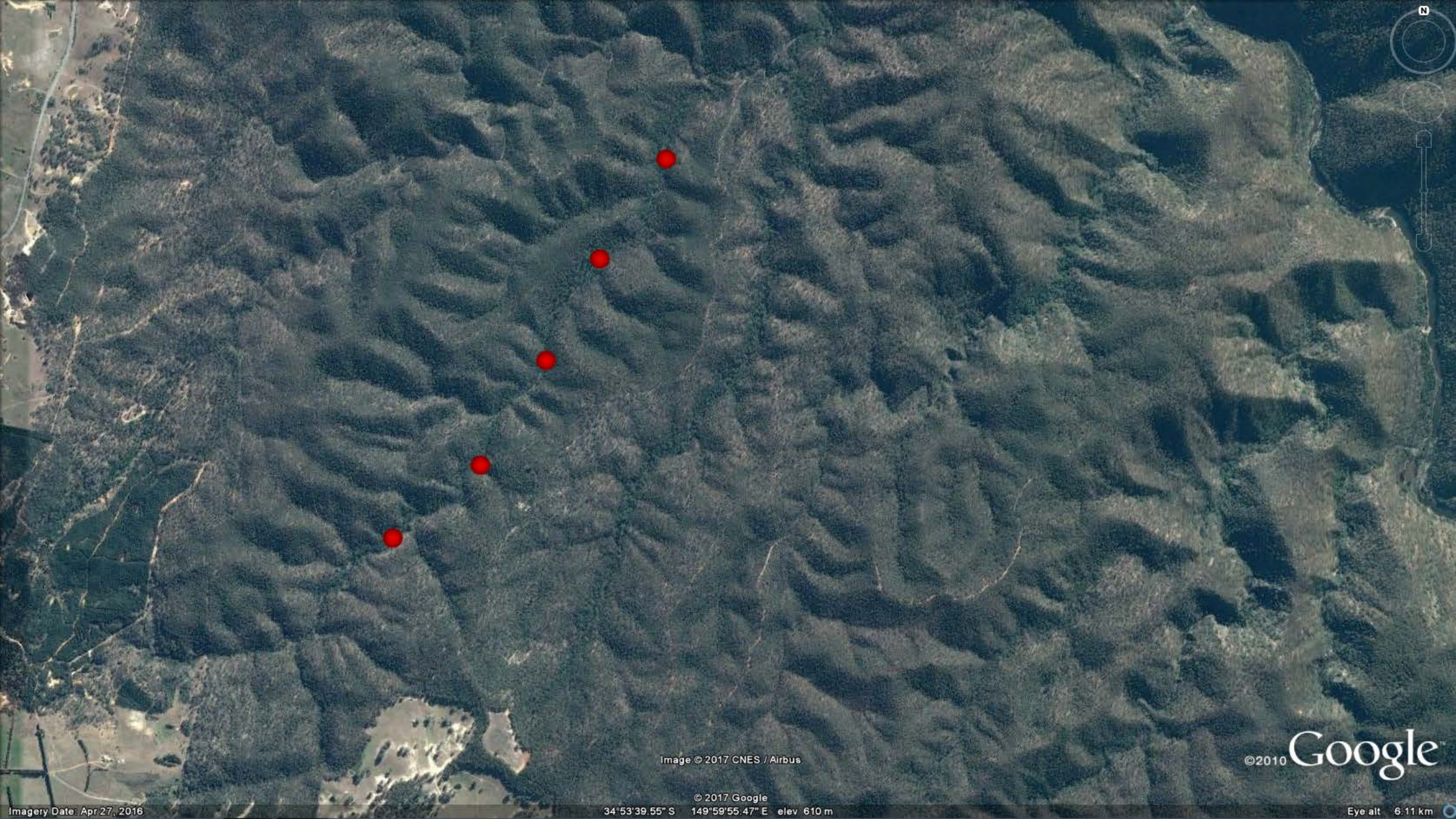
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34°53'39.55" S 149°59'55.47" E elev 610 m
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34°51'51.39" S 149°59'47.25" E elev 608 m
Eye alt 3.32 km

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The key elements - For more effective conservation collecting:

- Aim to sample from across the area covered by the population
- Using a minimum distance target between plants sampled
- Most critical - ensuring each accession / sample are collected from one plant and kept separately with a specific accession number

The key elements - For the users / collectors

- Employing a collection method that meets the requirements for delivering the purpose for collecting.
- Maximise the chance of a successful collection event with a protocol / checklist.
- Ensure each team is armed with a user friendly guide and reference to enable efficient and effective collecting.

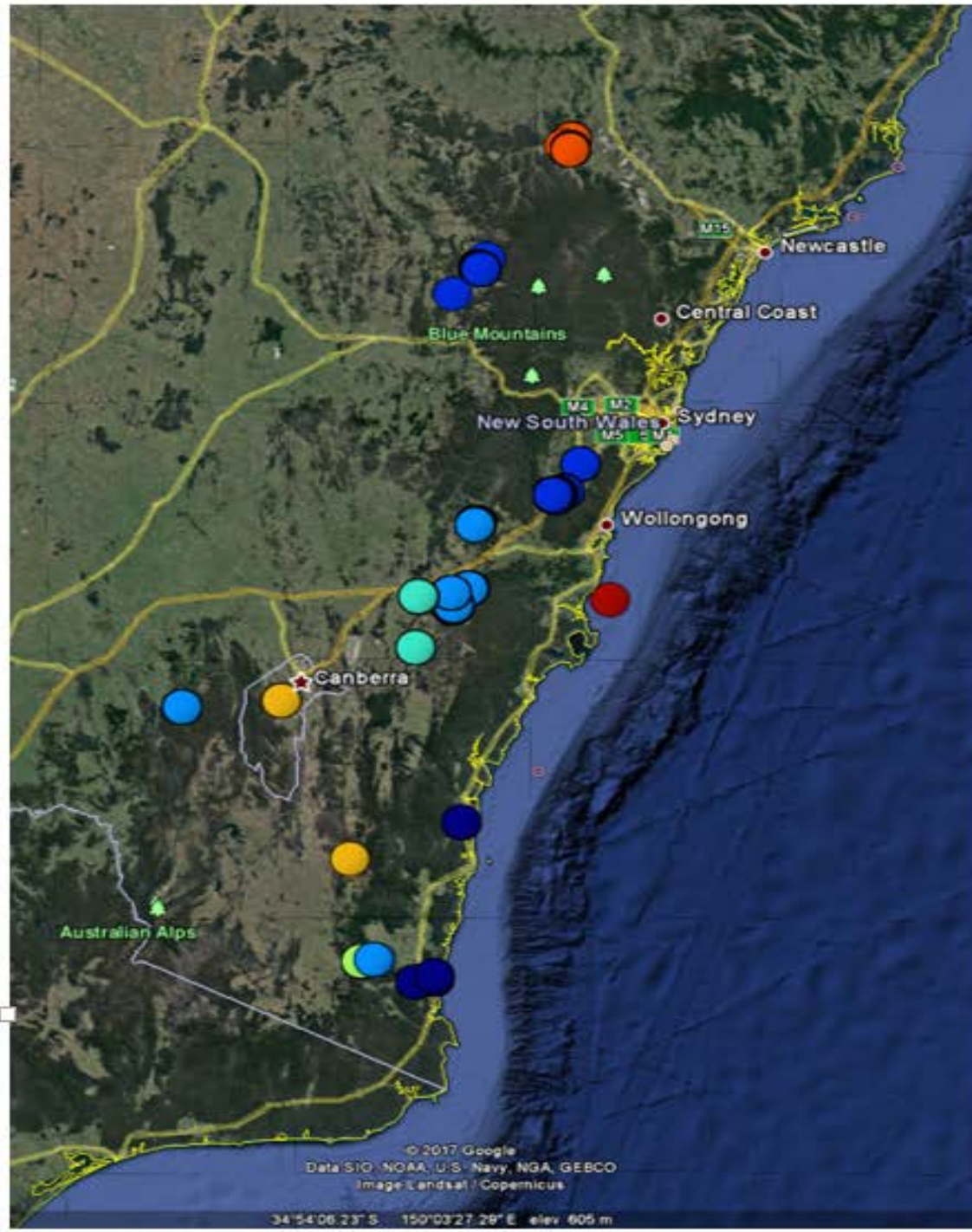
Collection summary

(May 2018)

Approximate total number of established cuttings, seeds collected, and number of putative populations (and sub-populations) sampled to date, per species.

	Species	Populations (sub-populations)	Cuttings	Seeds
NA	*P. adnata*	TBC	TBC	TBC
	P. bodalla	2 (1, 3)	170	8,300
	P. brunnea	2 (2, 3-6)	300	86,300
	P. cotoneaster	4 (3, 3, 1, 1)	250	135,800
	P. delicata	2 (1, 1)	350	9,500 (cultivated)
	P. elachophylla	1*	NA	31,200
	P. pallida	3 (1, 1, 1)	25	1,700
NA	*P. parrisiae*	2 (1, 1)*	NA	8,000
	P. reperta	1 (3)	130	71,700
	P. walshii	1^	80	NA

*seed only
^cutting only
NA = Not applicable



What happens to the cuttings and seeds?

Flow cytometry to detect polyploidy

1

Take fresh plant leaf

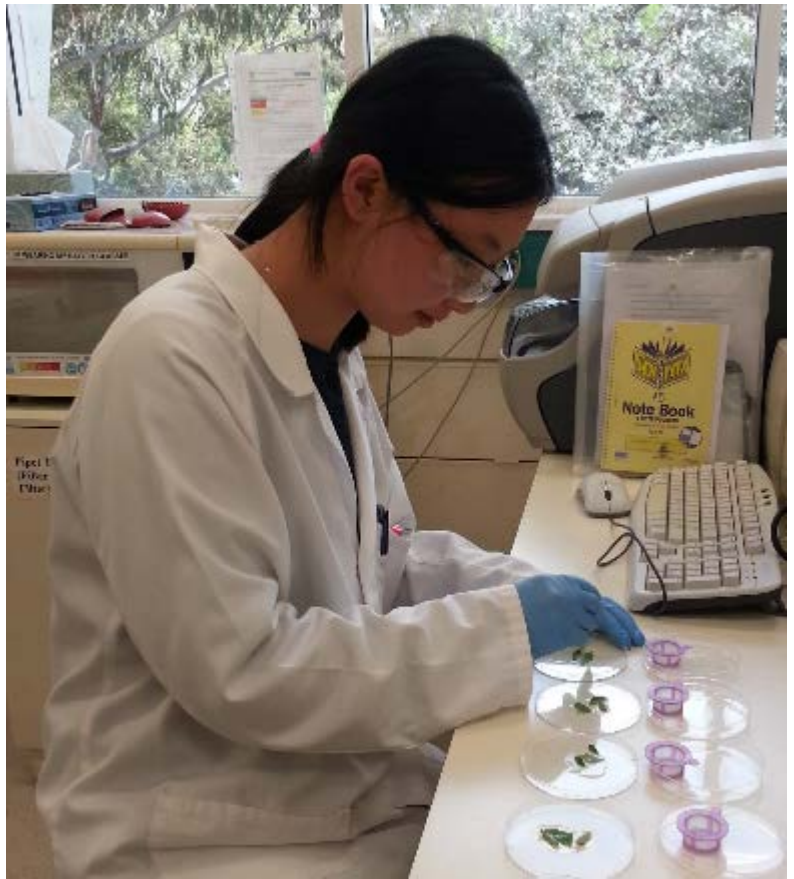
Release cell nuclei into buffer

Stain DNA

Measure sample against standard with known genome size

Calculate sample genome size from peak ratio

2



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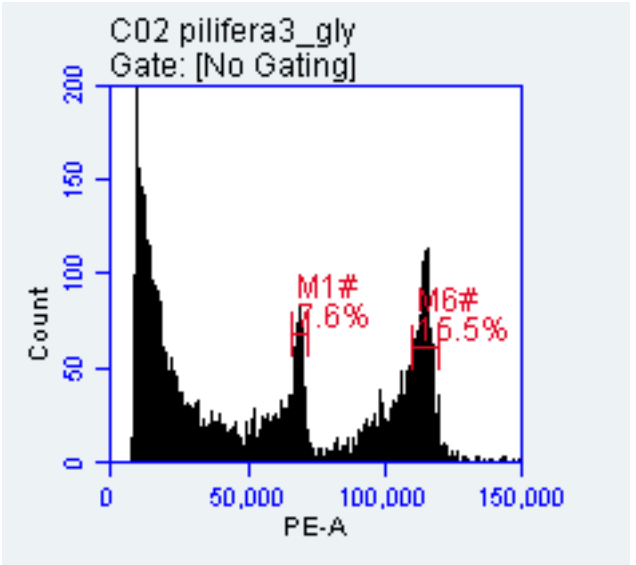
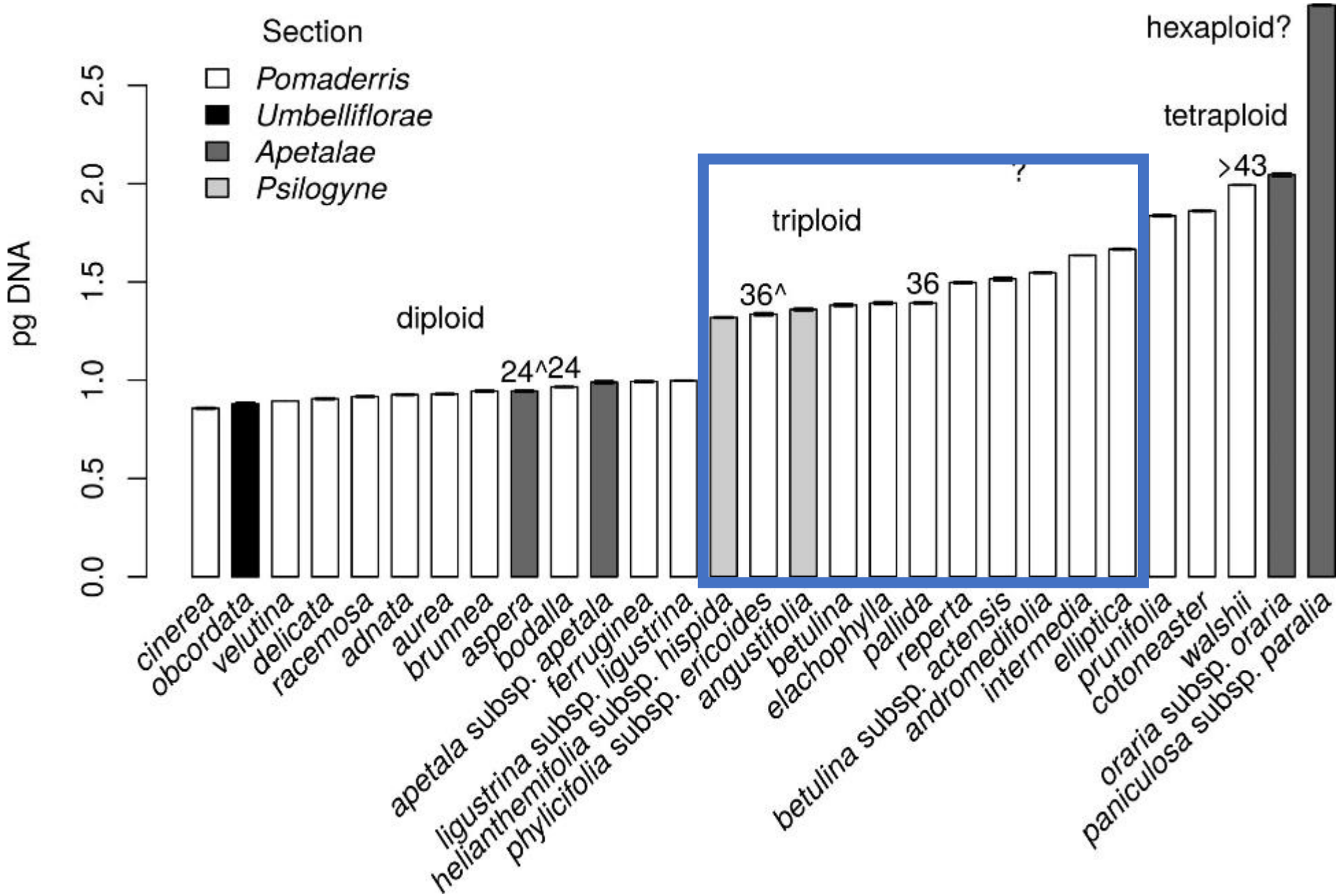


Within-species variation?

- Screening genome sizes to test for within-species variation
- No evidence for different ploidy levels within species
- ...but between species

Variation across species?

- Polyploidy rampant & most likely several independent origins



New question

- Triploids often sterile, but here entire species triploid
- How do the triploid species reproduce? (And how do the others?)
- New Zealand triploids apomictic,
  i.e. producing seeds asexually i.e. a clone
- → flow cytometric seed screen

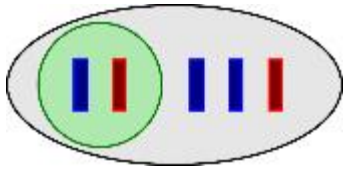
Likely reproductive method

Normal (2 sets) DNA

Normal reproduction, seedling is a mixture of mum and dad's genes

Sexually reproducing diploid (2:3)

- *P. cocoparrana**
- *P. lanigera*
- *P. ledifolia*
- *P. notata*
- *P. obcordata*
- *P. vellea*

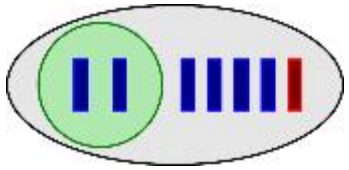


Normal (2 sets) DNA

Needs pollen but doesn't pass on those genes. Seedling = clone of mum

Pseudogamous apomictic diploid (2:5)

- *P. bodalla*
- *P. brunnea*
- *P. cocoparrana**
- *P. costata*
- *P. delicata*
- *P. discolor*
- *P. mediora*
- *P. velutina*

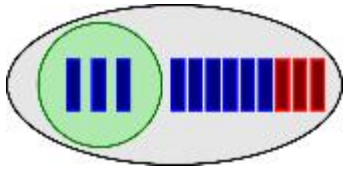


Triple (3 sets) DNA

Needs pollen but doesn't pass on those genes. Seedling = clone of mum

Pseudogamous apomictic triploid (3:9)

- *P. andromedifolia*
- *P. argophylla*
- *P. eriocephala*
- *P. intermedia*
- *P. pallida*
- *P. prunifolia*
- *P. queenslandica*
- *P. reperta*

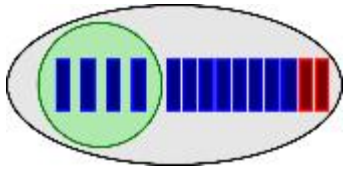


Four x (4 sets) DNA

Needs pollen but doesn't pass on those genes. Seedling = clone of mum

Pseudogamous apomictic tetraploid (4:10)

- *P. cotoneaster*
- *P. parrisiae*



Are the seeds any good?

P. lanigera
“Normal” sexual diploid
Photographer: Hall, W.

P. eriocephala
Asexual (clone) triploid
Photographer: Clinton, B.

P. intermedia
Asexual (clone) triploid
Photographer: Clinton, B.

Seed ecology project

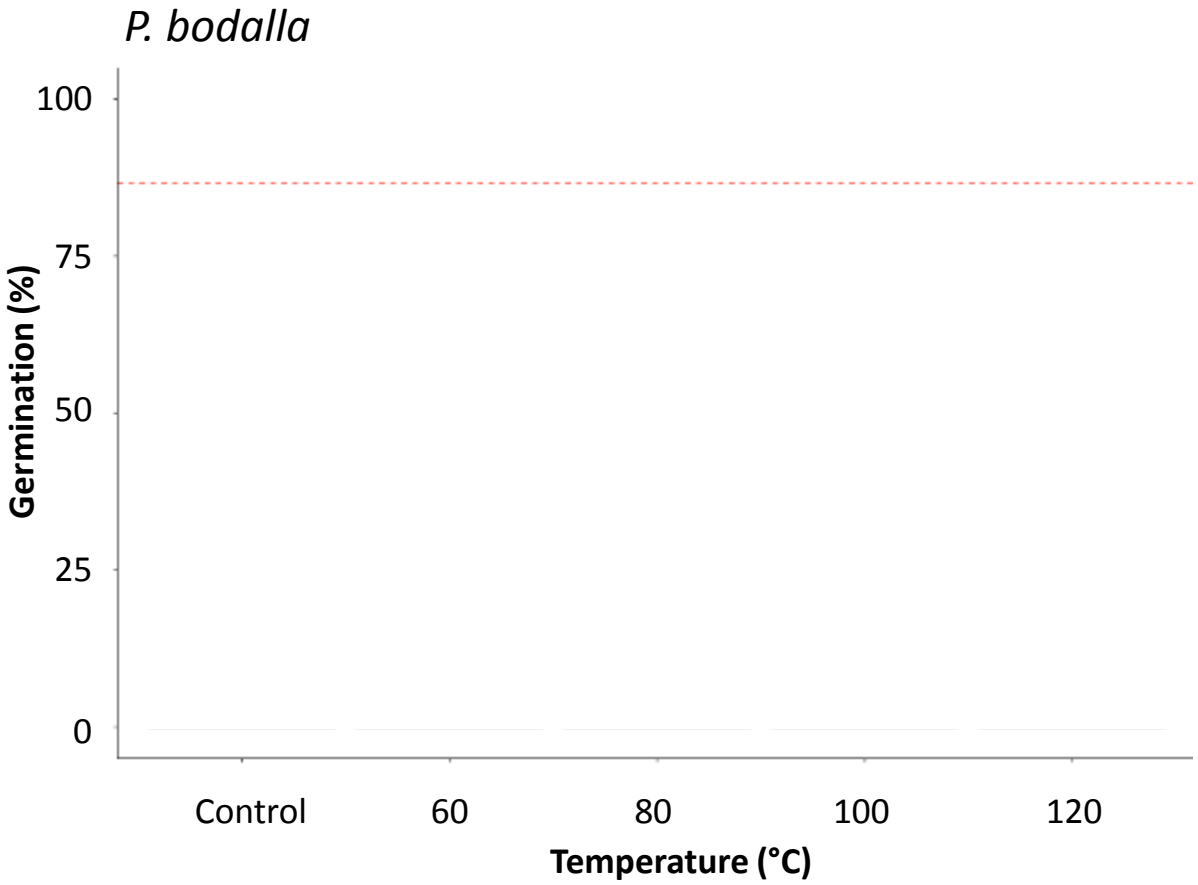
- Jason Chan, Hons, UNSW
- Does polyploidy influence seed and seedling traits of common and threatened *Pomaderris* species?
- Potential drivers of species rarity

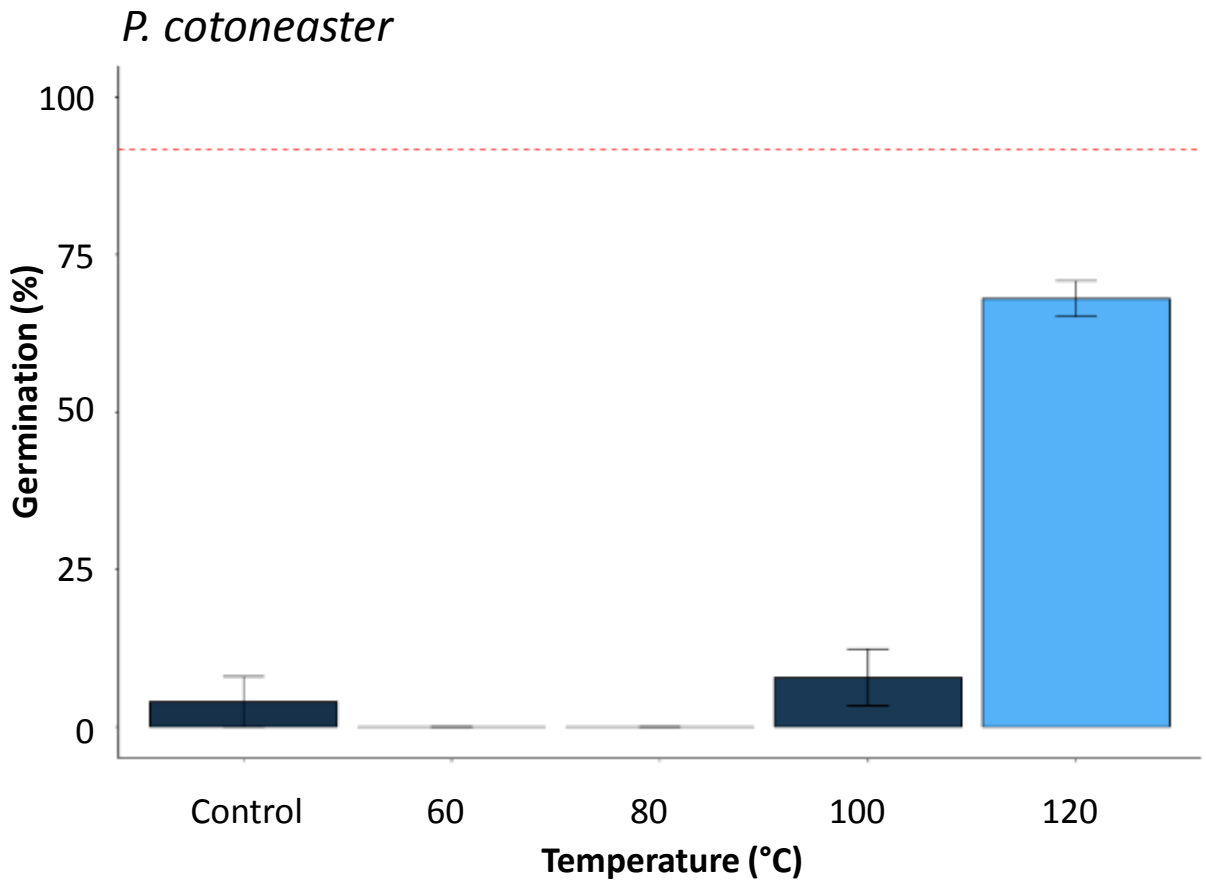
Physical dormancy

- Many Rhamnaceae, including *Pomaderris,* have physical dormancy
- Seed coat prevents water uptake and germination
- Usually alleviated by fire (or weathering)

Photo: Brook Clinton

Dormancy alleviation thresholds





120 °C is optimum for 10/11 species

Implications of the research results

Result	Implication
Within-species ploidy variation not detected (…yet)	Reproduction at population level not limited by ploidy variation
Species have at least 4 different ploidy levels (2, 3, 4 or maybe 6 sets of DNA)	Polyploidy is part of evolutionary history of the genus
For some species, very few plants produce seeds	Soil seed banks may be relatively small
Among individuals that do produce seeds, the numbers are highly variable	Mothers contribute unevenly to the next generation
Majority of species produce seeds that are clones of the mother plant, for triploids that helps avoid sterility issues	Probably low genetic diversity
Temperatures required to alleviate seed dormancy are very high	May not occur in many, especially riparian, habitats

Prone to localised extinction?

What has this got to do with genotype collecting and translocations?

GENOTYPE COLLECIONS Check-List	
Permit Number:	NSW OEH SL101586
field book number range for this genotype sequence :	JKS20 to JKS22
Accession Number range: To be completed by Database Staff:	**907312 - 907314**
HERBARIUM Voucher	
Field book number of the herbarium voucher/s:	JKS20
POPULATION SAMPLING Information	
No of Genotypes sampled	3
Field book number for genotypes sampled:	JKS20 to JKS22
Target minimum distance:	10 m
Estimated population size:	50-100 plants
estimated area of population :	c.180 m x 110 m
GENOTYPE COLLECTION Information	
Plant Number: (if applicable)	PC0199
No of plants collected from	1
Material collected (Cuttings/Seed/DNA /other)	Cuttings

Today has focussed on one option tailored for a specific project as an example.

The principles can be applied and tailored to any ex-situ conservation collecting. (Particularly for non-seed methods)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