Collections management – documenting conservation collections using a genotype collecting method

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Background

Many threatened plant species are typically grown from seed for *ex situ* security; for re-introductions, landscape enhancement, translocation and offset actions, with the aim of improving the trajectory of populations. They are also grown from seed for use in research to gain a better understanding of the species in question. However, propagation from seed is not always possible due to difficulties in obtaining viable seed and, if seed can be obtained, there is often a lack of information on whether it can be readily germinated, grown in cultivation and/or plants established from the seed collected.

When seed-based options are limited or there is a high uncertainty of the success of these methods, a practical alternative or complimentary method is the use of non-seed methods such as growing plants from cuttings or other vegetative means. Regardless of the horticultural methods adopted, the key challenge is to identify suitable population sources and to ensure resulting propagules have traceable links to parentage for future research and use in species recovery.

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 for research, plant production / seed orcharding and options for translocation or landscape enhancement. A genotype collecting method can be used to help address many of these issues.

A genotype collecting method – providing a tool for addressing the needs for strategic conservation collecting

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

- Be able to trace source populations and have control over genotype selection for future planting/re-introduction/translocation.
- Maximise the chance of a successful collection event and enable population 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, enabling more effective comparative analyses across projects and easier access to key standardised information and terminology.

The Genotype collecting method can be considered as two key elements:

1. The Principles that underpin genotype collecting

Genotype collecting or 'maternal genotype collecting' can best be defined as collections that are sourced from a population where multiple maternal genotypes (parent plants, rather than population aggregates) are sampled and accessioned individually. Single and multiple types of germplasm may be collected from an individual plant but are linked by an accession (typically a number or several numbers) to the specific parent plant and to all the subsequent germplasm secured *ex situ*.

This ensures that existing and future users of the germplasm (collected and secured *ex situ*), have access to material from a known source, linked to key information, and therefore ideally suited to:

- Translocations.
- Re-introductions.
- Landscape enhancement.
- Establishment of new populations and offsets.
- Seed orcharding and *ex situ* plant production.
- Research projects.

This approach provides the genetically representative ingredients that many restorative conservation objectives strive for including, separate and traceable maternal genotype collections.

Genotype collecting can be applied to both seed and vegetative (non-seed) collections, noting that although the seed collection is usually not one genotype, each accession is taken from only one maternal genotype

(parent plant). Using common terminology for a 'maternal genotype collection' will enable easier access to records and information in the future. This helps readily identify records where germplasm was collected from one maternal plant only as opposed to collections done as a mixed population sample (*i.e.,* those where vegetative or seed material from many individuals in a population are bulked to obtain a representative sample of unknown numbers of parent plants). Conservation projects that require adequate representation of the genetic diversity found in wild populations, and require control of that diversity is where the method comes into its own, not only by having various genotypes secured as separate germplasm. More importantly, it enables control of the genotypes when designing augmentation, reestablishment or translocation trials as the known genetic diversity underpins the method and ultimately improves the likelihood of success.

2. Maternal genotype collection protocol

This protocol is designed to clarify the collection size, lineage, collection method, and provide a link to key information (e.g., number of plants sampled, proportion of population sampled, linked accession numbers). Building upon the principles outlined in original guidelines for germplasm collection and translocation developed by the Australian Network for Plant Conservation (ANPC) (Offord and Meagher 2009; Commander *et al.* 2018), this protocol adopts a 'maternal genotype' sampling method for seed and non-seed collecting as a collection method and checklist. It outlines the key requirements to be considered when collecting living plant samples intended for reintroduction and has been developed with the aim to ensure all field collecting teams use a standard method.

Designing the protocol

The three key aims of standardising collection methods, capturing key information and ensuring lineages retained from source wild material, led to the evolution of two key components:

1. The field collecting and sampling method has to consider the number and size of plants in each population.
2. Each genotype collected needed to be traceable from the propagation and production phase through to translocation readiness.

Collection method checklist

When sampling using a genotype collecting method the following protocols should be used:

1. Establish the extent of the population. This should be the first step before collecting commences.
2. If feasible, make an estimate of the size of the population e.g., '<50 plants' or '50-100 plants' and over 200 m^2 etc. Noting this may not be practical or possible if the population covers a considerable distance/area and/or the vegetation is dense and of mixed species of similar height.
3. Once extent of the population is known, (or estimated), aim to sample plants
   a. from across this population
   b. determine a minimum distance between sampled plants ideally greater than 10 m (to reduce the chance of collecting closely related samples). Topography and extent of population can influence this distance.
4. Record the estimated minimum distance in the field book records for the collections. If tagging of sampled plants is an option, aim to tag an agreed number of plants with unique labels. This should be done in numeric order and ideally be spread out across the sample area.
5. Try to place the tag in a visible place as these plants may be re-visited in the future for re-collecting and research. An option to assist re-visiting the plants sampled is to attach a piece of flagging tape so that it is approximately 150 mm to 200 mm long. This helps to relocate the tags when re-visiting and re-collecting. If tags are not being used identification and location will be covered by each field book entry.
6. Collect a herbarium specimen containing flower or fruit.

When sampling material in the field:

Vegetative material:

1. Write a plant label; include the plant name and collection number (these can be pre written to save time in the field).
2. Place the label in the cutting bag. It is good practice to duplicate the collection number on the bag in case the label goes missing.
3. Take the cuttings.
4. Lightly mist the cuttings, seal the bag and avoid crushing during transportation. Keep as cool as possible and out of direct sunlight.

Seed:

1. Seed dispersal can be unreliable and we suggest bagging the developing seed and re-visiting to harvest.
2. Check pollination has occurred and early seed development is in progress. Avoid bagging flowers as this will prevent pollination and reduce seed set.
3. Place 3 to 4 bags (ideally) on a single plant.
4. The seed collection event is only recorded at harvest. This involves the creation of the field notes. It is always separate to the cutting field notes
5. To harvest; cut the bags from the plant and write the collection number on each bag.
6. Tie all the bags harvested from one plant together so it is clear they come from the same maternal genotype.

Considering timing and frequency of collecting

Carefully planning the timing and frequency of collections trips can have a huge impact on the likely success of a collection activity. Knowing as much as possible about the plant, the location and in particular the likely timing of flowering and seed set can make a huge difference.

When combining seed and non-seed options, consider conducting two or more trips to enable greatest chance of success, for example:

- First trip: Locating and assessing population, tagging of plants distributed across population, taking a herbarium specimen, collecting cuttings from the tagged plants, bagging fruit for follow up collection.
- Subsequent trip/s: Re-visit population and collect seed from bagged plants, re-collect cuttings if previous cuttings have not succeeded.

Field book sample for 'genotype collection' events – What your field book may look like

1st page of field book entry:

On the first entry for a population sequence, highlight the following two items above the normal fields. (These are in addition to the standard field book data/entries)

1. Use standard wording such as 'Maternal genotype collections' or 'genotype collections' to indicate the nature of the collecting method.
2. Indicate the number range of sampled collections from this population (e.g., JKS 20 – JKS 30) and the total number (e.g., 11 collections). Data items for this need to be determined after writing up all the collections made from one population on one day.

Note: There is no need to repeat this information for the rest of the population sequence, as the number range of collections (2 above) implies it is carried over.

Then continue with the Standard field book data:

Permit/Scientific License number: where applicable and in keeping with legislation relevant to the collecting event, the location and species being collected.

Collector and No.: using surname and all other initials if known (e.g., J.K. Smith, not J Smith)

Date and Country, State and District: fields should be completed at the start of the day and when they change, only.

Locality: either 'generalise' the locality for the whole population sequence (e.g., '1-2 km from road intersection') OR note that some details of locality change slightly through the sequence as GPS coordinates change, and record these changes.

Habitat: when moving from one collection to the next in the population (with GPS coordinates changing), please note any change to habitat, especially aspect, and make clear which data is carried over from one record to the next. For closely adjacent collections, usually everything is carried over and should not need to be rewritten.

Latitude, longitude and altitude: all fields should be completed using either grid reference or GPS. It is essential for Datum to be recorded in the first entry of a population sequence. Latitude and longitude should be recorded in degrees, minutes and decimal seconds.

2nd page of field book entry:

Plant description and notes:

- **A description of the plant**: including height and width, and any other significant information e.g., 'weeping form'
- **Area (or distance along a watercourse or road):** covered by the sampling of the population. Note this may not be the entire population area.
- **The target** (aimed-for) **minimum distance between plants sampled:** it does not need to be written on every entry, rather only at the start of each population sequence. There is no need to record the distance between individual plants actually sampled, as this can be estimated by mapping the points later (if required).
- **Estimate of the size of the population:** if practical e.g., '50-100 plants' etc.

Herbarium specimen: record which sample has a voucher from the population and any duplicates and their destination.

Record what was collected: including seed, cuttings or whole plant, and/or voucher.

Phenology: record whether voucher specimens (only) have bud, flower, fruit etc.

Template which flags to the collector factors to be included in pre-collecting planning e.g., Mt Imlay

MATERNAL GENOTYPE PRE-COLLECTION Check-List (Population)	
Population descriptor	
Permit No.	
POPULATION SAMPLING Information	
Estimated area of population	
Estimated population size	
Target minimum distance	
Target no. of plants to be sampled from this population	

Field book number range for the population and field book number(s) for population vouchers to be recorded in the field

Template insert for field books which flags to herbarium registrar the need for additional information to be added to each record in the prescribed sequence.

All other standard collection information still needs to be entered on each page of book particularly the item type of living material.

MATERNAL GENOTYPE COLLECTION (Population)	
Population descriptor	
Permit No.	
Field book number range for this sequence	
HERBARIUM Voucher	
Field book number(s) of the herbarium voucher(s) for this population	
POPULATION SAMPLING Information	
Estimated area of population	
Estimated population size	
Minimum sampling distance	
No. of plants sampled from this population	

The above method covers a conventional collecting scenario and can be used as a template or guide to edit and modify where needed on a case by case basis for each particular scenario.

Summary

This collection method can be applied and adapted for any species when the aim is to conduct translocations or reintroductions and where tracking of genotypes/ populations is desired. It is particularly valuable for non-seed collecting methods where the tracking and management of lineages can be a challenge and is often resource hungry. It also enables *ex situ* collections sourced using this method to provide a genetic representative and translocation applicable resource that can be tapped into for future conservation, research, and plant production programs.

The method has been applied and adapted to a range of conservation projects and implemented by many collection teams and across many jurisdictions and is available for use by all involved in the collection of flora for scientific and/or conservation purposes.

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- Australian National Botanic Gardens.
- Wollongong Botanic Gardens.
- Booderee Botanic Gardens.
- Eurobodalla Regional Botanic Gardens.
- Australian Botanic Garden Mt Annan.
- NSW Office of Environment and Heritage (Editors note: now Department of Planning, Industry and Environment).
- Illawarra Grevillea Park.

This group has pioneered much of the development, trial and initial implementation of the genotype collecting method.

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