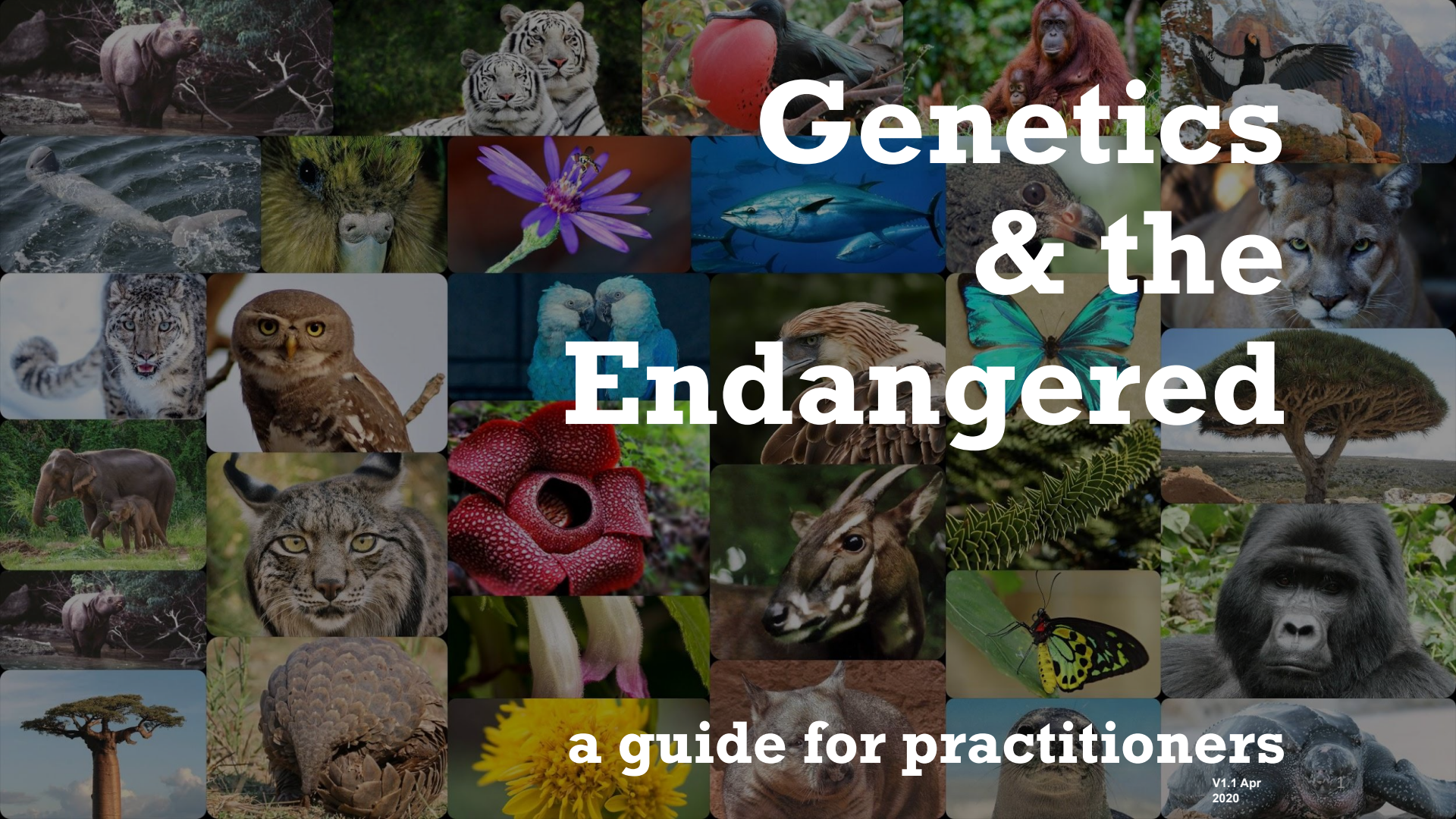

Genetics & the Endangered

a guide for practitioners

V1.1 Apr 2020

BRIEF: ReForest Now was funded by the Wettenhall Environment Trust in 2019 to complete two objectives under funding received.

Objective 1: Complete 2019. 1: Demonstrate the process of attaining a genetically diverse collection for an endangered species.

Objective 2: Produce a handbook for lay practitioners that describes the concepts and practical guidelines for working with endangered plants to maximise genetic fitness and likelihood of long term survival.

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on behalf of ReForest Now

Foreword

In the 21st century, conservation has become a priority on the global agenda. Environmental awareness enjoys rapidly increasing social currency with the power to influence political platforms and cripple corporations. When a movement comes to notoriety, however, that popularity will, by its very nature, always create discord as differing opinions, approaches and agendas clash. Often the sheer weight of conflicting evidence collated by vying corporate groups, expert bodies, and an increasingly activated, emotion-driven populace dictate the narrative. Unfortunately, conflict breeds scepticism and resistance, and so the cause is often forgotten amid the chaos, and this is never more true than in the field of conservation. Recent years have seen an ever-widening chasm emerge between the evolving evidentiary approach of genetic scientists who endorse abandoning endangered species in favour of vulnerable and threatened strands, and corporate conservation programs who achieve significant success in improving survivability outcomes by harnessing scientific methods only possible with significant funding. Finally, there are those working at the coal face of conservation: landcare managers, farmers, and dedicated environment-focused communities tackling the most difficult target groups: critically endangered species. We believe the future of conservation lies in the hands of this sector. The way forward is to provide those on the frontline with the necessary tools, concepts and solutions to facilitate their vital work without recourse to study, improbable funding, or strict abeyance to the checks and balances of the scientific realm. As you would all agree, you don't need a lab coat to understand the lay of the land. This booklet aims to give you a 'need to know' guide to conservation genetics and a strong foundation upon which to practice meaningful and far-reaching environmental stewardship. Working with species on the brink of extinction, the risk of failure is ever-present and undeniable. But the key to success is preparation, hard work and learning from your failures, and if this guide helps you do just that, then we are well on our way. Together.

What is this guide going to do for me?

ReForest Now has produced this resource for very specific outcomes. These are categorised in the two columns below.

What it does

Provides a basic overview of genetic science, endangered species and genetic diversity.

Teaches at a layman, basic level the underpinning concepts and understanding needed to have a sense for conservation genetics.

Enables the reader to have a stable basis from which to learn more about conservation genetics.

Provides several direct action tools at several stages of project management for conservation programs that are easily converted into immediate action for you.

Provides real scientific examples to support our suggestions.

Provides concepts derived from university trained, practicing scientists and their reference materials.

What it does not do

We do not generally provide scientific references or a high level of scientific teaching in this guide. That is beyond the scope of the document.

It does not cover working with **endangered animals**, although many principles apply and we still recommend that you read it and convert what you can for that purpose.

It does not support the dominant paradigm of some of the generally agreed ideas that have spread throughout the conservation community. We are especially in discord with the term ‘local’ provenance as ‘local’ has misled many laymen. Our reasons for diverting are explained with both concept explanations and our own real world examples from recent field and genetic research.

It will not teach you everything you need to know to do the best work you can. Question all assumptions and even question our recommendation, there are always exceptions, especially with endangered species.

Contents

1. Introduction

1. Introduction to genetics of the endangered

For any endangered species we work with, we must consider its history, how it survived over time and the environment where we now find it, this tells us a story.

On the genetic level, these stories are made up of a number of factors. Firstly, there are mutations in genetic material that push the species to change over time. There are also changes in opportunities for the species, which can come in various forms and are important in deciding a species destiny. These can be as follows;

- The formation of a new ecosystem niche (eg. rainfall increases in a lowland area, allowing wet-adapted species to thrive)
- A new vector for distribution of seed and pollen (eg. a new type of bat or bird that disperses seed)
- Threat due to external factors (eg. changes in the local landmass fracturing a population)
- Genetic losses (eg. a fire or a drought that kills a portion of the population and leads to a decrease in genetic diversity)

If we want to support adaptability of species to survive beyond our lifetime, a priority should be to ensure they have genetic diversity. Genetic diversity will maximise a species capacity to radiantly adapt to many unknown future opportunities and threats and to avoid inbreeding, genetic faults and species collapse.

As we head into a future with a changing climate, our species will have better chances of survival if they have gene pools that enable adaptation in times of; unprecedented drought, fire, flooding, spread of viruses and pathogens and changes in rainfall and ecosystem structure.

This booklet is the beginning of a “genetics for endangered species” series with the aim of implementing genetic knowledge into your restoration projects for the long-term benefit of the species under your care.

1. Defining Endangered

According to the International Union for Conservation of Nature (IUCN), there must be an **immediate risk of extinction.** Given that risk, there are then **5** categories of assessment:

1.

Serious population size reduction, measured over 10 or more years or 3 generations.
To be Critically Endangered, a species would have a loss of over 80% of its population in that time. **To be considered Endangered, a loss of 50-80%.**

2.

Geographic range / area of occupancy, measured in km^2. Fragmentation into smaller groups and a decline to either of these categories.

3.

Small population size and decline, measured by the number of **mature individuals**: projected and actual decline. **Critically Endangered is considered less than 250**, **Endangered, less than 2500** mature individuals.

Within fragmentation scenarios, **Critically Endangered is considered less than 50**, **Endangered, less than 250** mature individuals in each fragmented subpopulation.

4.

Very small or restricted population. Low numbers of mature individuals and a restricted area of occupancy. **Critically Endangered less than 50**, **Endangered, less than 250** mature individuals.

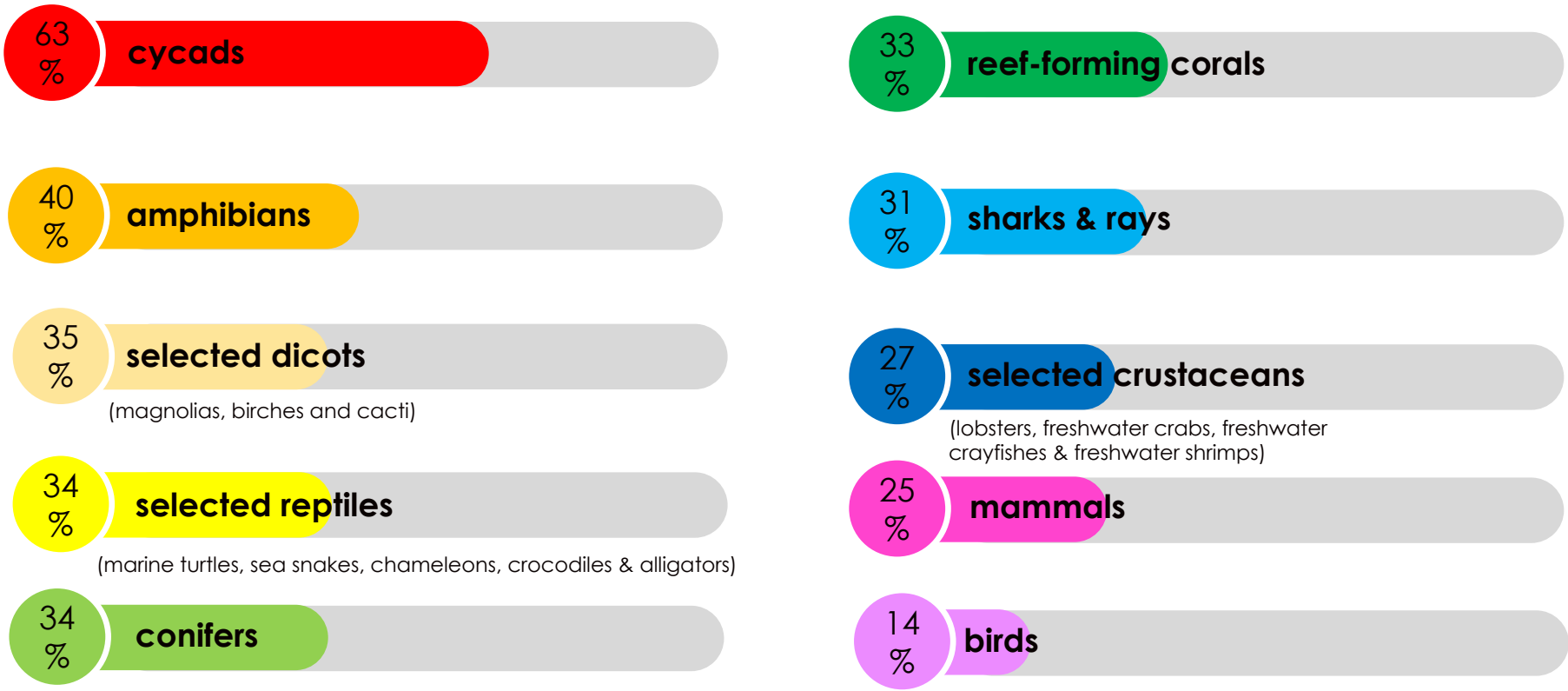
5.

Quantitative analysis. Specifically, the probability of extinction in the wild by percentage chance over time.

Critically Endangered is considered over 50% chance in 10 years or 3 generations. **Endangered, over 20% in 20 years or 5 generations**.

IUCN RED LIST: Guidelines for Using the IUCN Red List Categories and Criteria

1. Global percentages of endangered life forms



IUCN RED LIST: Guidelines for Using the IUCN Red List Categories and Criteria

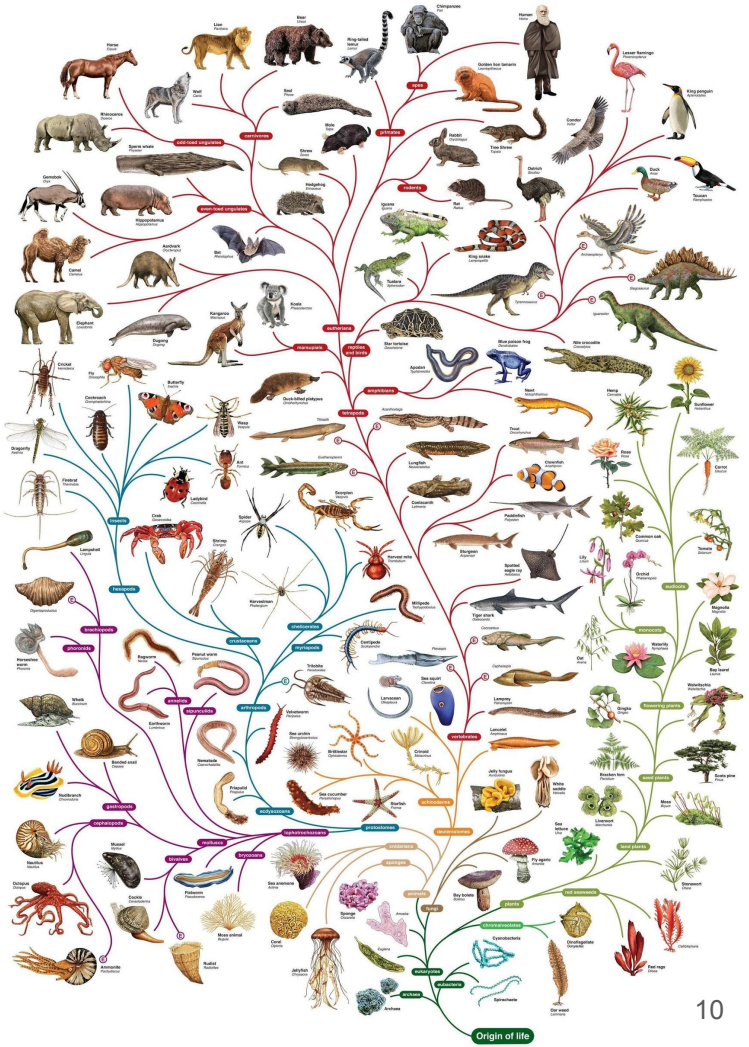
1. Genetic Diversity - What, why and how

What is a species?

Before we can identify genetic diversity, we must understand what a species is. A species is a group of organisms that are very similar at the genetic level. These organisms can exchange genes with one another in one of several forms of breeding, depending on several factors including where they are on the **tree of life**. The **tree of life**, also called the **phylogenetic** tree, shows us the connectivity and origins of all life on Earth, tracking back all evolutionary paths to common ancestors billions of years ago. Cellular life is estimated to have begun about 3.8 billion years ago. Any group of Earthly organisms belongs to a branch of the **tree of life**. We are all related. The specific classification is then as follows from the basic to the specific:

- **Domain** (type of cellular structure)
- **Kingdom** (plants, animals, fungi, etc)
- **Phylum** (basic structure of organism)
- **Class** (mammal, reptile, etc)
- **Order** (specific subtype of class)
- **Family** (specific subtype of order)
- **Genus** (first part of latin name) i.e. Homo
- **Species** (second part of latin name) i.e. sapiens

A specific type of life form, **a species,** will have the most genetic material in common with those other species closest to it on the tree of life. In this way, slight changes in the genes of a species push it away from its nearby relatives in new directions. Those relatives too are not stagnant; they also change and evolve, and so the tree of life has always been growing and expanding away from it's common root.



1. Genetic Diversity - What, why and how

What is a species?

More specifically, here we are looking at plants. Moving down from the **Kingdom** level in the diagram, you can see that more and more branches diverge off and less species belong as the level becomes more specific. By the **Genus** level, there are only around 500 organisms in the group of *Rosa*. Basically, *Rosa gallica* should only breed with other *Rosa gallica*. However, in all species there exists the opportunity to breed at times with genetically similar organisms from the same **Genus**, but different **Species**. In this instance the species has been interbred for hundreds of years with others in the *Rosa* genus and sourcing a pure genetic specimen may no longer be possible. When it comes to endangered species many programs are very concerned about avoiding this. **It's called hybridization**. Organisms exchanging genes with others within their own genus but from a different species can experience negative effects, such as infertility, low birth rate, or other issues you would normally expected from inbreeding. Hybrids are not considered part of the species and are at risk of being accidentally created by mismanaged conservation programs that can lead to loss of an endangered species. However, this is not simply a bad or good outcome in all cases - at times hybridization may be the only option in saving a species.

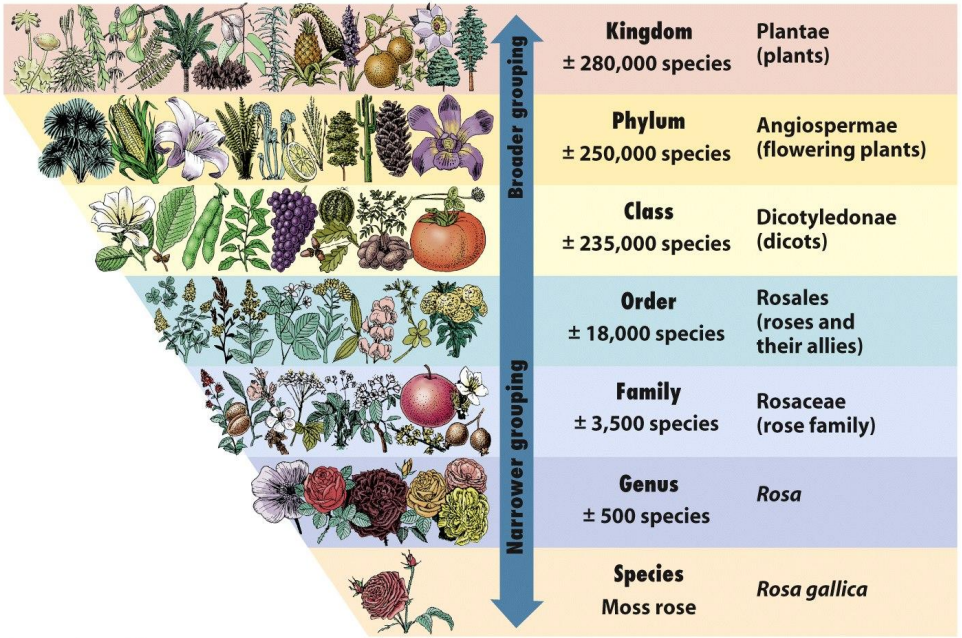


Figure 2-6 Discover Biology 3/e

1. Genetic Diversity - What, why and how

What is genetic diversity?

Genetic diversity is the possession of any genetic differences by a group of organisms of the same species.

We will use ***Homo sapiens*** as our example. ***Homo*** is the genus, ***sapiens*** is the species. We consider genetic diversity as any differences in the coding sequence that make up that species. In our case, we have 3.2 billion sequences of DNA information. The genetic diversity of humans is low - we have on average 1 in 1,000 of these basic DNA sequences differing from each other. That 1 in 1,000 difference between individual humans in our 3.2 billion genes - **that is our genetic diversity**. The differences are far less than 1 in 1,000 in families and other close knit relatives.

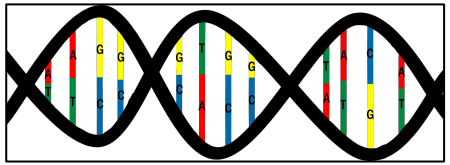
Those differences in our DNA are sometimes in parts of our DNA that write the code to make our physical bodies. When these DNA codes are called on by the body for building information, they are used to produce our body materials, and thus differences become visible. For example, when two people of different origins suntan, their DNA produces more melanin from the DNA code for melanin. Based on slight differences in the DNA code between individual humans, that melanin could be yellowish, brownish, or other colours. This is an example of a visible genetic difference: many parts of genetic diversity are not visible at all however, such as differences in immune function.

Differing amounts of two types of melanin (eumelanin and pheomelanin) dictate eye, skin, hair and genital colouring in humans.

1. Genetic Diversity - What, why and how

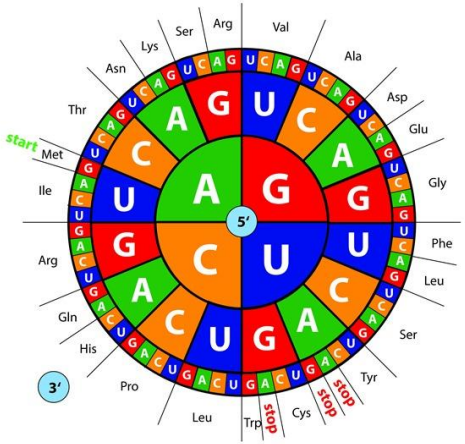
1.
The DNA of plants and animals is made up of 4 codes. The DNA of plants is usually between 800 000 and a few billion of these codes long and the entire sequence is kept in the nucleus of almost every cell in most plants. The DNA materials A go with T and G go with C. The DNA is only read one way, so it is important which way around a pair of A & T or G and C are oriented in on the top and bottom strands .

DNA Sequence



2.
These DNA codes (A&T, G&C) are copied by small molecules and brought to a 'printer' that reads the above codes in sets of three, called **Codons**. These tell the printer to select a single amino acid from its inventory and start building. The **Codons** are used to allocate one amino acid at a time, and make long chains of the amino acids. These are strung together one at a time, like a string of pearls. These long chains of amino acids are called proteins and go on to make up the materials of our bodies. Differences in codes here would make for differences in immune function or hair colour, for example.

Codon Wheel



3.
Here's an example:
Let's imagine this DNA sequence on the left is a small gene. Read from left to right in sets of three (**codons**) on the top line and then look below.

AAG, GGT, GGT, ACA

This is raw DNA information. It needs to be converted into a message before anything else - this is almost the same, but T's are substituted with U's.

We then have: AAG, GG**U**, GG**U**, ACA.

4.
Read from the middle of the wheel for the first letter, then out for the second and again with the third letter. Then add the letters from above.

AAG = **A center**, **A second tier** and **G third tier** = '**Lys**' = Print the amino acid **Lysine**.

GGU, **G center**, **G second tier** and **U third tier** = '**Gly**' = Print the amino acid **Glycine**.

1. Genetic Diversity - What, why and how

Why is genetic diversity important for the survival of endangered species?

The small differences between us allow for a range of different responses to living in the natural world - and offer differing and unexpected benefits. Sometimes an organism's individual chance to survive is improved by being large and able to store enough calories to survive a long winter. Other times, being small could mean being able to live in an environment with less food availability all year round. Sometimes having a robust immune response protects from certain viruses, but at other times, an overly strong immune response could be the cause of death. There is no single fittest way to be. In this way, genetic diversity allows us to radiate into opportunities or be resilient against myriad adversities, not just as individuals, **more importantly** as a group. When some of your species survives an event, the species survives an event, there's no single 'hero format'.

When a species is endangered, it is almost certain to be low in genetic diversity, due to a variety of possible causes. Simply, assuming your organism carries two versions of each gene (which many organisms do), 50 surviving organisms of one species may perhaps only carry **an absolutely maximum of 100 versions of each gene**. In reality this variation is far less, as there are always many genes that are identical between many or all individuals. For example, a person might be twice as tall as another (there being a broad genetic diversity for height), but virtually all people have four fingers and a thumb on each hand (there being low to no genetic diversity for digits).

Though genetic diversity varies based on many factors such as historical events, population size, fragmentation, and disease exposure, we may expect that many genes across individuals are the same, with only some differences - for example, in humans only 1 in 1,000 genes present a difference. For endangered species this is even more critical. We should begin with the assumption that out of a surviving population, there exists only a handful of genetic differences. This is because we know that not every gene is going to be different between two individuals, and that being an endangered species, there are not many individuals left to carry many genetic variants.

These last survivors must be protected at all costs. There are so many risks of being low in genetic diversity that any further losses should be seen as unacceptable.

1. Genetic Diversity - What, why and how

Why is low diversity dangerous?

1. A species of low genetic diversity has a lower chance of evolving to adapt to changes in their environment, increasing their risk of extinction. For example, a species of 400,000 individuals may carry a variety of genes, perhaps including one for drought resistance. In a severe drought event they may be reduced to 15,000 survivors who carried that gene and the species continues, whereas a species with just 50 survivors is far less likely to carry a portfolio of genes that allow for some of its individuals to survive an environmental pressure. In this sense, a single stressor (like a drought) that affects all members of the species negatively could cause extinction in a single event - if no individuals in the species have a way to survive that specific event.

2. Inbreeding - this is breeding between closely related individuals. While it sometimes occurs as a part of some species norms, it does lead to groups of low genetic diversity that are vulnerable to single stressor events, as detailed above. Inbreeding tends to lead further towards the loss of group genetic diversity, especially over several generations.

3. Inbreeding depression is an even more serious issue. This can happen when dysfunctional versions of genes that were previously uncommon become common due to chance, small population size, inbreeding, or a combination of these factors. These dysfunctional genes generally only become active when there is no alternative version of the gene present in the individual - they will only activate if there's two copies of them and nothing else.
   For example, a species might carry a rare version of a gene that makes up 15% of the gene pool and causes blindness. This gene is usually spread thin across the species. Only occasionally does an individual inherit two copies of the blind gene and cause a blind individual to occur. We call these recessive genes because they are usually unused when any other copy for that genetic sequence is available. Specifically, these harmful genes are called **recessive deleterious genes**. Other recessive genes are not harmful at all, such as the gene for red hair.

1. Example: Inbreeding and Extinction

In this example, we will imagine a palm species with both male and female plants that carry two genes that can be deleterious when two copies are inherited. This gives no option to the DNA but to use the ineffective gene.

With only three survivors left of the species, this palm is forced to undergo inbreeding. The recessive weakened anti bacterial resistance allows their bacterial pest an easy kill (two pale blue genes). Those born with two defective copies of the germination gene are unlikely to germinate. Within three generations the species is extinct (two copies of orange).

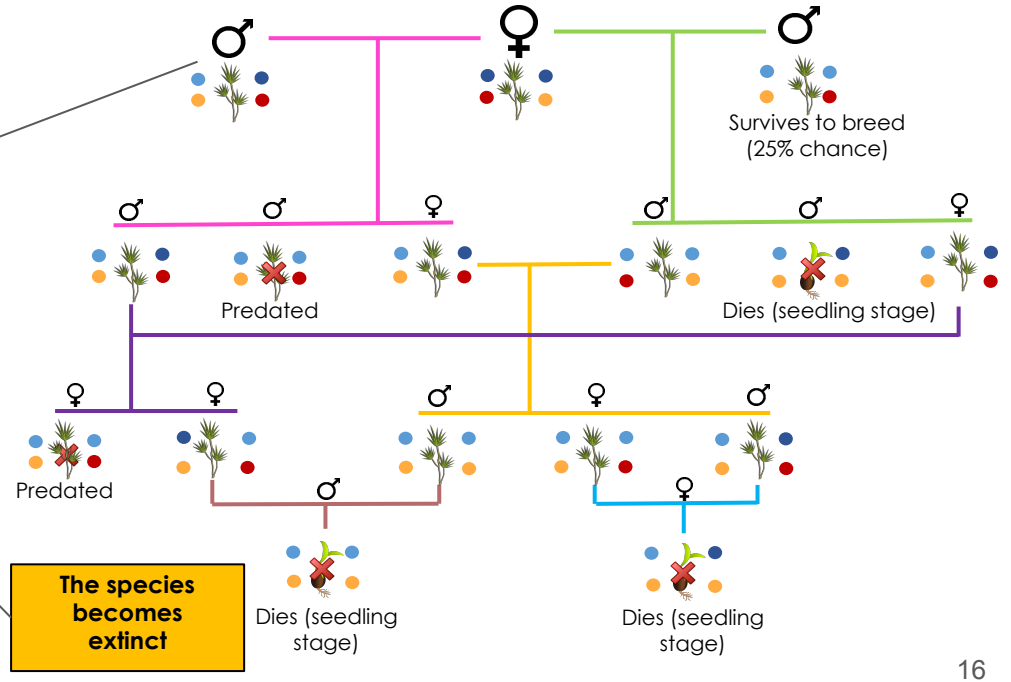
In such a scenario only genetic management could save this species. The probabilities of losing the species to a scenario as shown is too high. If managed for long enough and to a large enough population size, new mutations will slowly accumulate again.

GENE 1: Dominant
Anti bacterial resistance gene.

GENE 1: Recessive
Weak anti bacterial resistance gene (75% chance of being predated on before breeding).

GENE 2: Dominant
Healthy germination gene.

GENE 2: Recessive
Poor germination gene.



1. Example: Recessive genes

When reports indicate that a species suffers from inbreeding depression, it could present as a number of symptoms, including but not limited to:

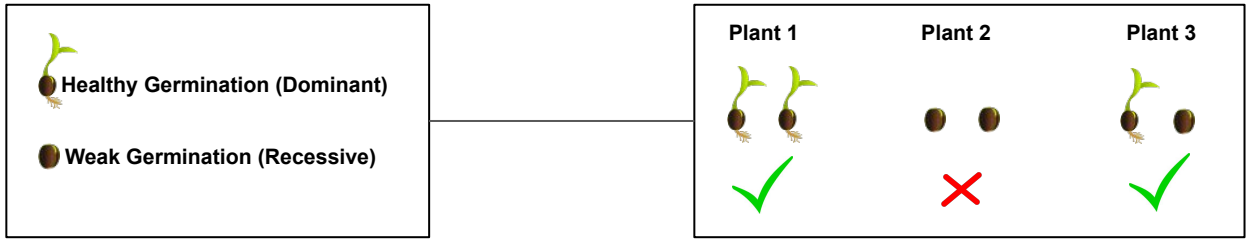
- weak growth
- failure to produce fruits
- early death
- poor weather resistance
- poor germination rate

If a plant has **recessive deleterious genes** for seed germination, it has only the same copies of one version of the gene that codes for germination and that code is not functioning well. Many species have two copies of every gene. If one copy is a recessive, it won't affect the plant, as long as it has a dominant healthy gene as well.

Plant 1 had two copies of the dominant gene for healthy germination and thrives.

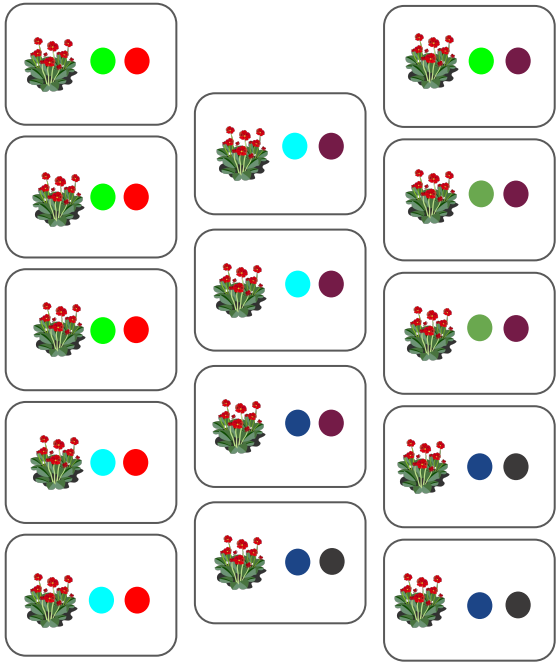
Plant 2 has two copies of the weak germination gene, which is recessive, so it's seed germinate very poorly/not at all.

Plant 3 carries one copy of each gene, but because the healthy germination gene is dominant, it fruits just as well as Plant 1.



1. Example: Genetic bottleneck

When a species have recovered in number from a near extinction event, they may appear to recover and carry a larger population, that is in fact low in diversity. Consider that, many organisms only carry 2 pairs of each of their genes and that many of those pairs can both be the same version of that gene. We know this can become a problem if the pair of same genes (recessive genes) are dysfunctional (recessive deleterious genes). Being a pair of recessive genes itself it not strictly a problem, deleterious recessive genes are. Here, we give an imaginary example of a highly harvested medicinal herb. It was reduced to just 14 individuals by 1900. With recovery efforts there are now 100, 000. For ease of understanding, let's imagine it has 2 genes.



From our 14 survivors, we do not expect every single one to be completely different to the next genetically. We expect to see some similarity and some differences. Here we note **gene 1** and **gene 2 (left and right on the plants)**. The versions of gene 1 are; green, blue, light blue, dark green or dark blue. The versions of gene 2 are pink, red, marone, or black. Each version of gene 1 or 2 make up a total percentage of the gene pool.

Green : 4 / 14th

Light Blue : 4 / 14th

Dark Green : 2 / 14th

Dark Blue : 4 / 14th

Gene 1

Red : 5 / 14th

Marone : 6 / 14th

Black : 3 / 14th

Gene 2

When the population is bred up to **100, 000** in just 120 years, we expect that very little new mutation has occurred. However, not zero. The more individuals there are, the higher the chances that one will mutate a new genetic variant *(it's like everyone buying a lottery ticket to their own private lottery - the odds don't go down when more people buy tickets)*. Therefore, our populations genes may look as below. This should be a serious warning to those working in conservation to consider what could happen to an apparently recovered species if hit by threat. That small gene pool could be exposed and an apparently saved species, could later be exposed to rapid population loss by its genetic bottleneck of the past. This could be due to single stressors, such as viruses or drought that could kill off any of these variants in single events.

Gene 1 Green : 4 / 14th (28, 570 carriers)

Gene 1 Light Blue : 4 / 14th (28, 570 carriers)

Gene 1 Dark Green : 2 / 14th (14, 285 carriers)

Gene 1 Dark Blue : 4 / 14th (28, 570 carriers)

Gene 1

Gene 2 Red : 5 / 14th (35, 714 carriers)

Gene 2 Pink NEW : 1 / 14th (7, 142 carriers)

Gene 2 Marone : 5 / 14th (35, 714 carriers)

Gene 2 Black : 3 / 14th (21, 428 carriers)

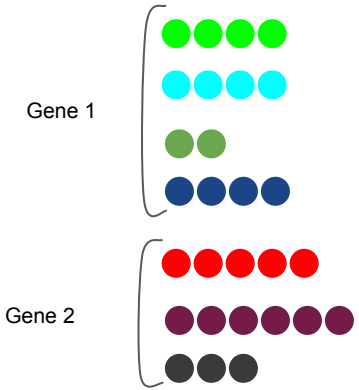
Gene 2

1. Example: Genetic bottleneck - visualised

To simplify the concept, lets display the genes as the ratio they exist in.

Original 14 survivors ratio of genes

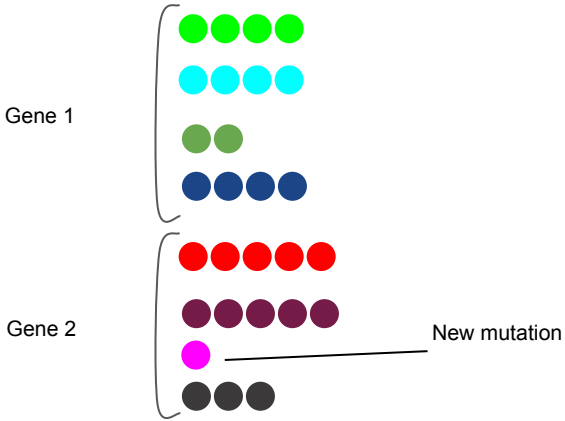
Here we see a display of the two genes that our 14 surviving trees carry. There are 28 genes here in total. Note this page shows the **gene pool**, not the individual plants. (The genes in their plant are demonstrated in the last page).



Four gene variants for **gene 1** and three gene variants for **gene 2.**

Ratio of genes 120 years later

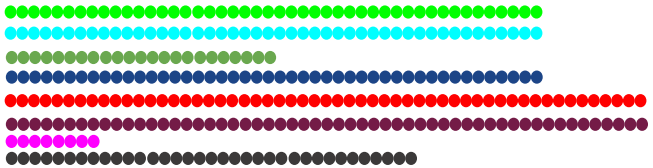
The ratio of genes is almost the same after 120 years of breeding of the survivors. Note that marone reduce slightly in frequency as pink has appeared. This is due to the fact that all new mutations are mutations of an existing gene. Little has changed in 120 years, bar one new mutation in one gene.



Four gene variants for **gene 1** and four gene variants for **gene 2** after 120 years of large scale breeding.

Expanded view of 'recovered' population ratio of genes

As a visual exercise we have extrapolated the ratio of genes in the population out to imagine a slightly larger group, *(but we won't try to show you what 100,000 would look like)*. As you see, we appear to have saved the species from extinction, but it is very much still vulnerable due to low diversity risks. Imagine that the red and marone versions of gene 2 makes the plant vulnerable to a tree virus. If just those two versions of gene 2 fail **71, 000 will be lost -** 7/10 th of the species *(refer to carrier rates on the last page)*. Low diversity is a serious survival threat.



In our 100, 000 plants in our 'recovered' population, no matter how many there are, the genetic basis is still from just those 14 originals. There are lots of copies of the same few genetic variants. Easily lost to single events that could further reduce the gene pool and lead to extinction. Not every threat is here in this decade!

1. Survival of endangered species

How will genetic care help your species survive?

We've seen how environmental risks are high for a species with low diversity and that inbreeding and inbreeding depression represent survival risks. Generally, we can say that moderate or high genetic diversity represents higher health and less overt problems than we see with inbreeding depression. However, avoiding the negatives of low genetic diversity is not the only important point here.

Higher genetic diversity in the group and better spread out genes in the group and the individual organism also have benefits beyond avoiding basic genetic problems. We note that heightened fitness is found in creatures that have less pairs of general recessive genes. Remember that deleterious pairs of recessive genes *(the gene version that are negative and requires two copies to switch on)* are obviously harmful, while others have an effect that is neither negative nor positive *(such as having red hair), there is also simply that less general recessive genes seems to be a good thing, though it's almost impossible to study as many tiny negatives are hard to track.*

When working with endangered species we are often unknowingly mating inbred individuals from fragmented populations. These have often been breeding with limited numbers of their species for some time and may have no overt problems at all *(no obvious recessive gene issues)*. However, when mating from two inbred but healthy individuals that have genetic differences **between different** populations, we find their offspring can display greater health and more variety in physiology (like body shape or colour) than either parent or parent population.

That interesting effect can be explained: some inbreeding depression effects are large and obvious, while some effects are not. When we increase diversity we find **undetectably small problems disappear** at the overall performance level of the organism, and it then performs even better than a normal individual from its species.

2. Species assessment

2. Assessing your species I

Using science and experience to assess your species

If your species is well known, it may be well studied. No overall management rules and guidelines can ever specifically cover all situations for all species. As such, we would recommend having your team investigate the following assessments before planning any recovery program.

A. Was the species recently reduced in number? How few were there left? A species that has recently become low in number is far more at risk of suffering escalating genetic issues than one that has sustained low diversity for a long time *(probably being one of the species without serious genetic issues hidden in their gene pool)*.

B. What is the current population size? If the population contains over 4,000 - 4,500 individuals that interbreed and are not cut off from each other, the chances are that their genetics are acceptable and meet the minimum expectations of several conservation genetics authors. If not, start mapping and assessing the numbers remaining, where populations are smallest and what numbers remain in those locations.

C. Has their habitat changed or been reduced recently? Ecological relationships are far less understood than biology itself. A somewhat small change in a habitat, let alone a large change, can make it no longer suitable for a species that may have lived there for a long time. Sudden retraction of habitat area can also cause sudden species loss as many species require unexpectedly large areas to sustain them over all seasonal periods, food and for adequate breeding opportunities *(think ecology)*. These retractions can cause unexpected fragmentation. **What is fragmentation?** It is when a species is separated by broken habitat and can't connect as well as before or at all. Consider what happens in a family that doesn't live close together, can't see each other and can't communicate via the internet or phone, before long those that are cut off from the others are living as if their family are never coming back or don't really exist anymore. Fragmentation can make individuals from your species live as if they are alone, as if the other fragmented groups don't exist at all. Inbreeding can happen very quickly and unexpectedly if this has not been noticed. With less insects, birds, bats and other seed distributors and pollinators than ever before, the chances that seed or pollen are being distributed between fragments is lower than ever.

2. Assessing your species II

D. Has any genetic study or other literature been published or otherwise released regarding your species? Are there differences in diversity at different populations? Where from? Do subspecies exist? Some academic work may require that you have access to a system like Scopus, Web of Science, or other to obtain full records.

E. What have other conservation programs done? What were their considerations and methods? Were specimens translocated? Direct contact is always recommended with like minded organisations that have done work with your species.

F. Who are the world's leading researchers for this species? Direct, clear, questions with your case being presented as having a clear tangible outcome are most likely to be returned with an informative email from an expert. Contact may need to be attempted several times with busy academic or conservation professionals as your email is almost certainly a low priority.

G. Do any populations of your species carry disease? How would you avoid spreading that? What risks does it pose to the composition of the population? How might that affect your approach in the future?

H. Contact seed collectors, identification book authors, forest regenerators and nurserymen. These people may have anecdotal or written knowledge not available on google or in the mainstream. Listen out for stories of clusters of your species that never fruit, although they are seen year after year, or for other signs something is wrong.

I. Look for direct physical clues of inbreeding. You may discover species specific and known faults from the above vectors of information gathering yourself. These are described simply on the next page. Listed are some of the known types of problems we see in plants with inbreeding depression.

2. Assessing your species cont'd

What can you look for in your species to determine if the species might be inbred?

Increased disease susceptibility from viral, bacteria and fungal vectors

Unusual appearance of leaves, flowers, stems etc (straying from the normal characteristics, deformed or underdeveloped)

Lower insect diversity found on the species than known to inhabit it and known to be ecologically present

Lowered robustness of bodily form

Increased predation on leaves, stems and roots

Failure to flower, or low amounts of flowers produced

Poor temperature resilience (within species weakness to heat or cold)

Failure at the sprouting stage for many/most/all seedlings

Failure to reach maturity, premature death or collapse

Failure/reduced capacity to produce species specific natural compounds (including colouration or phytochemicals)

Failure to produce seed, low yield or partially developed seed embryo

3. Seed collection

3. Considerations for seed collection I

What should you consider before collecting seeds?
Distribution of genetic material is affected by a number of factors:

- **Seed distribution range**
- **Pollinator range**
- **Barriers in the landscape** (such as mountains)
- **Global events** (major changes in climate, or major weather systems)
- **Local events** (such as fire or fragmentation)

These are variables that change markedly for different species. Unfortunately, for most plant species we have incomplete data on the ecological relationships and previous natural connectivity. In regards to pollinators and distributors, they are often unknown/partially known. For your species, we recommend researching in detail to discover all that is known. Some questions that you can begin with include;

A) What colour are the flowers? White? (these may be insect distributed, perhaps discluding; birds, bats and insects with UV vision). You may be looking for a beetle, for example and you would want to consider its natural pollination range. Understanding pollination range of a species is step 1. Are flowers brightly coloured, UV patterns or not? (These may include UV sensitive organisms).

B) Is the fruit fleshy and edible? How large is the seed? Could it be transported by local birds, bats or rodents? Look for evidence of consumption of fruit while out in the field as we as literature and anecdotes from the industry leaders in your area (these become textbooks eventually).

C) Is the seed too large for birds or bats? It may be water or rodent distributed, rodents can explain water distributed seeds found far from creeks.

3. Considerations for seed collection II

What to consider before collecting seeds

D) If the seed is **self** distributed, what kind of mechanism is it? Is the plant spore based? What moist air vectors exist in the local microclimate that would be successful? Consider fragmentation, creek lines and other effectors of air moisture. Is it wind dispersed? How far can it travel? If it is water distributed, how far can it distribute from the creek/river or how far down-stream or into tributaries that only flow in floods? Older remnant trees can exist in unexpected locations and distributions and test your expectations.

E) What was the former range of the species in the literature? Are your collection sites really separate? Or just recently made so?

F) Does any genetic research exist on the species? If so, this may show you the true divergence of genetic diversity over distance and inform your collection practices accordingly. Some species might show high specificity to local microclimates that have emerged, these may show large genetic changes over 200 kilometres. Others might slightly change over 500 kilometres!

G) What traits does the species display in terms of its adaptability to habitat? What different types of microclimates is it found in?

H) What geographic boundaries exist that, definitely and actually, contain the species from moving across them. Is it found on both sides of the mountain range? On both sides of the river?

I) How many surviving trees were there during times of contraction or loss? Depending on your location this question might be better answered by recent historical records, ancient history or far older changes in the ecosystem (we will give an example of this later).

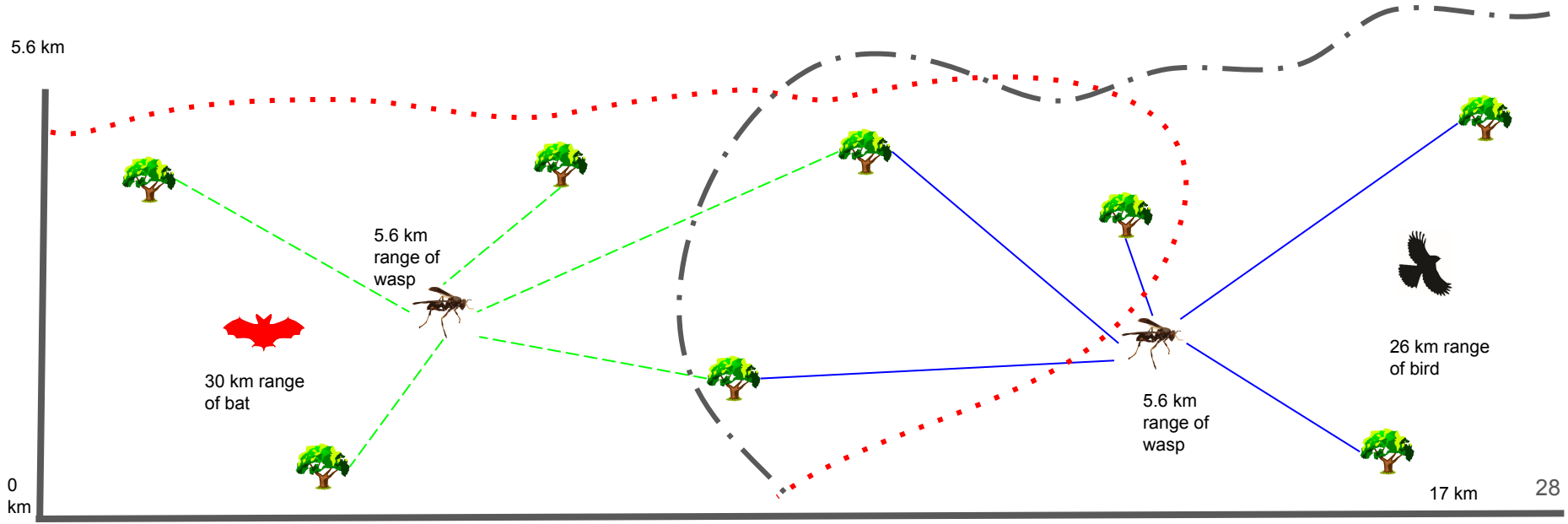
If you can answer these questions, you will know your species; seed and pollen distribution ranges, what distributes those materials, what non animal factors are important in distribution, the true reason why it exists why it does where it does, what microclimates it prefers or can handle, how fragmented it is likely to be, what is a realistic collection range to preserve and also maintain its genetic integrity, what landscape level ‘barriers’ are real or just perceived.

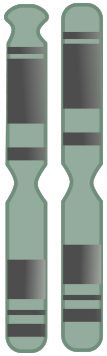
3. Considerations for seed collection III - Distribution and Pollination

Re-thinking local provenance - Traditionally our work in conservation has used the 'precautionary principle'. We've been cautious to collect seeds and seedlings only from a small local area where we are planting and have traditionally preferred local seeds with the advice this is beneficial for the local ecosystem. Local provenance seed collection has merit, however, it has been negated by many scientific publications,as an overgrown concept. This is because public discussion has inflated it to point where science has been forced to counter it.

Research in conservation genetics has demonstrated that 'local' gene pools are often much larger across a landscape than we think, the local extends farther and wider than we imagine to be local. E.g. for example, **birds** or **bats** can carry seeds a few hundred kilometers in a single day and pollinators such as **wasps/wasps** may travel 5 or more km in a single day spreading the local gene pool across large distances.

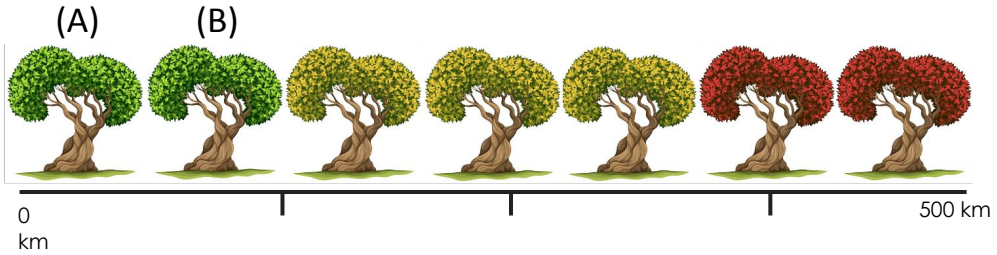
If this is what cross pollination and seed dispersal can look like in a day or a week, what will distribution look like over 1, 000s of years, as genetic material moves across the stepping stones of its species? The next few slides will continue on this theme of local provenance by distribution.





Genetic differences are noted in species distributed over very large distances. At times those differences can occur over reasonably small distances. It again depends on how the species is pollinated and distributed. It even depends on how genetically stable the species is. Some are stable for millions of years and thus distribution is less important an indicator of collection radius. All of these concepts have outliers, all endangered species need our best research efforts on a case by case basis. There is no one size fits all.

3. Considerations for seed collection IV - Relatedness by Range 1



Species that occur over very large distances tend to genetically vary along those ranges, sometimes to the point of **speciation**, where the genus remains the same, but a new species emerges at the end of the range. Imagine this as new genetic mutations happening at one end that find most of their breeding opportunities regionally, thus setting a new type in those at the far ends.

This example uses colour to simplistically show a genetic cline over a distance (*a cline is a gradient in genetic material, getting more and more different from one end to the other, as represented by green to yellow to red*). In humans, you could imagine this as the difference in appearance you might see in southern, darker people of Indian descent to those paler in the north, that is a cline. In this example, **Arbus verda** (on the left) becomes so different at the end of it's range it is red.

Genetic gradients over these distances are common, however as it applies to endangered species - local extinctions (*extirpation*) can occur when we take local collection to be more important than having a base of survivor trees beyond a few hundred, or arguably a few thousand.

When we try to preserve say the last stands of A and B, where there are only 50 and 30 survivors respectively, we are potentially dooming them to short term extinction, via genetic degradation or local events that could easily kill off just tens of trees with few responses to threat. Where low numbers occur there is always a risk of this. Better by far, to cross breed with the yellow populations just 125 kilometres away assuming that it has been ascertained that the species and genus are taxonomy certain and are the same (they are all Arbus verda). While this approach may seem novel in Australia, it is being practiced widely in the scientific world at large to good results. We are in this way, behind the rest of the world.

In this example, a winged seed (samara) is naturally distributed up to 100 meters from the parent tree

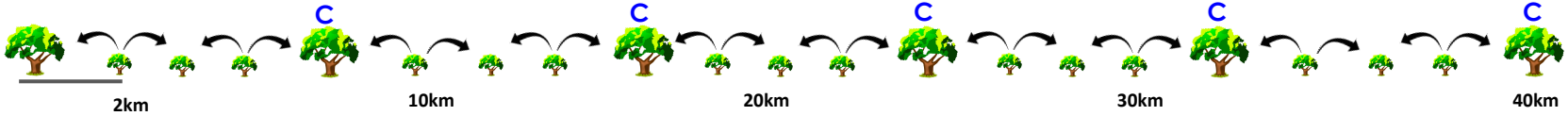
3. Considerations for seed collection V - Relatedness by Range 2

Wind blown seed over distance (ignoring pollinator travel)

1.

Seed is distributed just 100 meters from this original host tree. After just 5 years the tree labelled **B** has matured enough to seed and also has sent its seed a further 100 meters. In just 4 cycles over ~20 years it has been passed 400 meters. In ~200 years at this rate, the seed will have travelled 4, 000 meters from the source. In 2,000 years (*no time at all for evolution*), the seed will have travelled 40, 000 meters. 40 Kilometers.

2.



In the second example, we show seed distribution with **an image of a tree per 10 trees** that each distribute seed by a further 100 meters. Seed **is travelling in both directions** and there are likely **other mature trees across fragments** (shown with a **C**) surviving. Over distances relative to the distribution method of seeds, clines in genetics develop over tens/hundreds of thousands or millions of years. These should be respected in general over hundreds of kilometres. However – those distances and segregation are further than most peoples beliefs of local provenance. In this example, survivor trees are just 40 kilometers apart, in similar ecological conditions, they should not have distinct genetic differences that contribute to their adaptability to the local environment, **genetic differences may be detected**, but assuming they have local effects is unreasonable in similar site conditions and distances such as these. Assuming it is all structured cleverly is just colourful storytelling. Generally, we need to keep genetic diversity high in fragments much more than we need to preserve 'local genetic types' when it comes to the endangered. That changes only when research proves that two fragmented groups over such a short range have distinct genetic differences that have actual genetic applications to the organism (*not just random samples of the genome*). Essentially, we are often looking at **now** separate forests, that were genetically not separate at all for hundreds of thousands of years, this is due to all the factors we've discussed and more. Humans have created fragmentation and it is a vector of slow death for some of our endangered species, we need not exacerbate fragmentation with further flawed thinking about preserving 'local types'. Think of the genetic cohesion, when seed is being passed back and forward over the 40 kilometer range, if changes take hundreds of thousands of years, how many times will seed have been passed back and forward by then? Now consider weather events that distribute wind blown seeds further than 100 meters per seed season!

3. Considerations for seed collection VI - making things worse

The following compilation are known issues which impact seed collection relative to endangered species.

A) Forcing local inbreeding by 'local provenance'. Over zealous local provenance can mean collecting from too few and too closely related parent trees. This speeds up inbreeding and worsens the fragmentation and island effects. We must consider that when we collect from our local area we may be limiting the potential gene pool. Consider everything we've written when establishing your minimum collection range. Existing fragmentation means an existing period of time with increased shorter range breeding. You may be collecting from a species that is locally inbreeding already, it may need nothing less than more local genes to breed with. Be cautious what you consider to be a landscape barrier. Get on the ground evidence before taking anything at face value. It may not be the mountain cutting off seed flow between two groups, but local groups collecting seed on either side (that would otherwise be distributed by animals), breeding up local seed and keeping it local, that inbreeds the species. This can also give the false impression of genetic separation between two regions (man made local types and inbreeding).

B) Collection from street trees and easily spotted/known trees. Street trees were collected from either planted or remnant trees originally. They came from a nursery and many other trees will be coming from those same nurseries and seed collectors can only memorise so many tree locations or have the time to collect from so many. We are all guilty of collection of easily accessed trees. Over years, this can mean that a great deal of seed has come from one large, easily spotted specimen that several people attain seed from year after year. It is a trap. Flooding the gene pool of your species from a few individuals is a fast way to cause inbreeding. If you do collect from street trees, minimise it in place for those in the deeper, harder to get to places, with the least history of collection by others. Seed collectors can't reasonably go the extra mile for every species, as seed collectors for hundreds of species, but for endangered species, it is a must.

C) Creating hybrids from taxonomically uncertain or understudied species. This is a serious contraindication against several of our suggestions in the unlikely case that you are dealing with a species that actually consists of two or more species that haven't been identified by science and is thought to be just one species. You may find yourself eliminating a local type by increasing your collection range to overlap where two distinct types have actually formed. While it is often **not** the case that a local type is significantly different to other populations 100 or 200 kms away, there are times when they are different subspecies. The only thing one can do, is determine if there are any noticeable differences in appearance or function between your two populations or see if any genetic literature has recently determined a sub-species (as recently occured with south Sumatran orangutans *Pongo tapanuliensis* being identified in 2017 for example). There are always exceptions to rules, you will have to do your best research on a case by case basis.

3. Considerations for seed collection VII - making things worse cont'd

D) Gene flooding. Consider that there might be 100 individuals of an endangered species left and a breeding program then sources a lot of seed from 10 known trees and breeds 500 trees from that. These are grown on and planted in the local environment. The 500 planted trees come from 10% of the species 100 survivors. 5 out of 6 planted trees, plus the 10 parent trees they came from. These 10 trees seed collection trees are representing the genes of 510 out of 600 trees now. Does this sound like a path to genetic issues? Yes, It is. Always consider the number of parent trees by the total number of survivors and ensure you do not flood the gene pool by over growing from too narrow a seed source.

E) Spread of disease. Do your best to ensure that any known bacteria, viral or fungal diseases are known to you before you begin collection for your species. Several species have demonstrated pathogen resistance compromise when low in genetic diversity, unfortunate examples in the literature are abundant in the animal and plant kingdoms and tremendous failures have resulted around the world where careless introduction of disease have killed or all but ended a species on the edge. This is a big one.

F) Bulk collection. This will be spoken against again and again in this document. Never, collect more than small fraction of your total collection from a single parent tree. One should never leave any parent tree with kilograms of seeds. This point applies more widely than just in endangered species conservation. Global reforestation organizations are now planting trees by the millions, several reports have emerged showing frightening trends in massive bulk collections from stands of abundant trees (and closely related trees as well). It will not take long for this to cause significant genetic problems for these regenerating forests. Please support others by discouraging bulk collection as a practice in all situations.

G) The end of remnant trees. Collect from the oldest remnants trees alive today while they are alive. This means that trees that are survivors of the expansion of the human population from 1.5 billion to 7.5 billion people must be sampled as they have the greatest genetic integrity left. As conservationists tasked with helping our endangered species, we are always one of handful of people, or a handful of groups that work with that species actively in our lifetimes. Do not fail to source the best genetic material to give your species the best chance before those old remnant trees are gone. If we consider that 100 or 200 years is a long life even for many long lived species, we are at the closing period for a great many of these older trees that survived the outcome of the industrial revolution. The unique opportunity to retain those old genes is **now in our time**, a few generations on from us will not have the same chance, that wave of old remnant trees will be gone forever.

3. Seed collection case study I

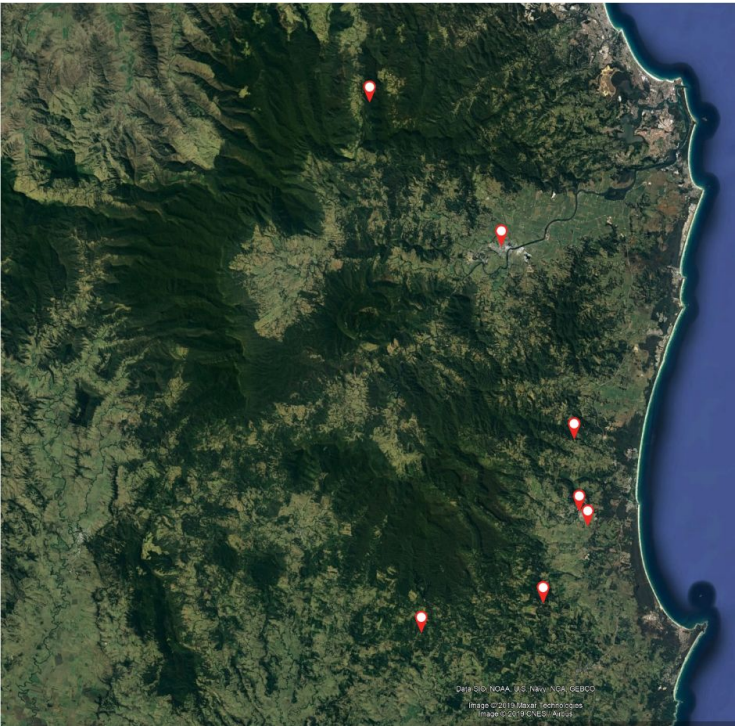
The Small Leaved Tamarind acted as our subject for a difficult demonstration of genetically diverse collection, as the species suffers from several challenges in surviving long term. It is recognised as endangered by the EPBC, NCA and BCA (Nationally and in both QLD and NSW). The species was reduced to perhaps 104 survivors across 20 locations in New South Wales and 5 locations in South East Queensland when it was assessed in 2004 (Stewart & McKinley 2004 cited in NSW DECCW 2004h). Each site consists of less than 20 adults, with only nine sites actively recruiting seedlings and juveniles. Sourcing genetically variable seeds under these conditions is challenging, as often is the case working with endangered species. The primary challenges recognised by the Australian government include; clearing and fragmentation and risk of local extinction due to small population size. We at ReForest Now have collected from fruiting trees at 7 locations, 6 in NSW and 1 in SEQ, working under the best conditions possible within regulations, known fruiting sites and availability.

(Above) A *Diploglottis campbellii* AKA Small Leaved Tamarind fruits. Despite being quite agreeable and non toxic to eat, it is unclear what consumes the fruit. Other Diploglottis species or Sapindales are often consumed by both birds and bats.

Inbreeding has been suggested as a possible end to the species. Inability to set fruits has been hypothesized to be the result of inbreeding depression as well as some individuals displaying weak growth habit. A degree of inbreeding is normal in many species, however, we've discussed that inbreeding depression is different. It is a state in which genetic diversity is very low and ineffective genes are forced to be active. If only 9 of 25 original sites set fruit then we can assume a genetic sequence causing this issue is being replicated without alternatives and is driving the species to extinction. The species also produces large and prolific fruit that germinate easily within 1 to 4 weeks and quickly develop to 10 cms above ground height within a few further weeks. This has made germination easy, but leaves concerns for viability in the seed bank under deforestation conditions. Rapid and effective germination quickly depletes them from the seed bank. Because of this, it may have been unable to recover from deforestation of the 1850s-1880s as it does not leave seeds dormant for longer periods than the above 1-4 weeks. This issue alone may be the reason the species is now endangered given the environmental issues it has faced. Further, many of the rainforest trees we call remnant trees, are in fact first generation re-colonisers and are less than 150 years old, other species may have been better off due to longer rests in the seed bank, therefore when we find 104 survivors of this species, one must ask, how many really survived the logging of that time if these are not the original survivors, but the offspring? There may have been even less than 104 survivors.

(Right) Juvenile leaves of D.Campbellii.

3. Seed collection case study II



Our search area included sites parallel with Byron Bay in NNSW and went as far as Lamington National Park in SEQ.

(Left) Our species collection radius occurred within the boxed region across the coastal NSW/QLD border. We attempted to cover most of the estimated range in which the Australian Government assumes to be suitable habitat and sought specimens over a area of 1020 square kilometres to ensure ‘a wide net’ approach to genetic sampling. Looking at this map, consider fragmented plant populations are left with the risk of being unable to share genetic material with close enough members of their own species, as pollinators and distributors can travel only so far between all this agricultural clearing. They must seek tentative habitat between forest patches and many pollinators rely on pheromonal cues to guide that may not be sensed over such long distances. Repeated close range breeding of a species low in number can easily result in inbreeding depression as is hypothesised in this species. By collecting over a range of over 100 kilometres we are likely to have sampled from trees holding some genetic differences (if any remain at all). We expect genetic diversity does remain in the species as some plants fruit and others cannot, some grow weakly, others grow healthily. Referring to the collection radius of 1020 square kilometres and 100 kilometres from furthest specimens (North to South).
What benefits could this wide collection bring to the species?

1. To sample genes that are only present in one location **‘private genes’**.
2. To capture genetic material from specimens that contain fewer genetic similarities that cause inbreeding problems.
3. To re-establish the flow of genetic material across the landscape as it would have occurred naturally.
4. To grow a larger and genetically healthy meta-population that can survive and mutate to create new variants for the future (higher numbers, more chance of new mutations).
5. To reduce the risk of losing a local population entirely through a single threat (fire, drought, etc) and the ‘private genes’ that would be lost with them.
6. Avoid flooding the gene pool with low diversity, high volume plants.

3. Seed collection case study III

1. Capture of ‘Private genes’: Referring to the map on the previous page, note that most of the species was wiped out and must have existed throughout the region at a previous time. From here we can assume that some genetic sequences survived only in perhaps one location (imagine frost tolerance or disease resistance). Without genetic testing we simply do not know what genetic value a single group may have to contribute to its species. For this very reason, we should always assume that any survivor of an endangered species, particularly those further from the center of the population potentially carry genetic value to its species that is only present in itself/its local group. Not only will the reconnection between fragmented groups allow for less inbreeding, it allows for the survival of genetic diversity and potentially the survival and integration of a valuable strain within a species. A strain that may well save the species from challenges or extinction later on *Please view the linked video from the 28 minute mark to learn more about private alleles in our research in an example from the closely related Diploglottis australis*- from actual genetic sequencing.
https://www.youtube.com/watch?v=5nJ6KUzmEzY

Imaginary example below: Imagine that we have entirely sequenced the DNA of the species and found the below positive traits of interest. In our own genetic research (*linked above*), we observed private genetic sequences in a species of the same same genus, found between fragmented populations just 30 kilometres apart.


Healthy growth habit


Anti-fungal extract


High germination rate

Frost tolerance


Drought resistance


Viral resistance


Northern population traits

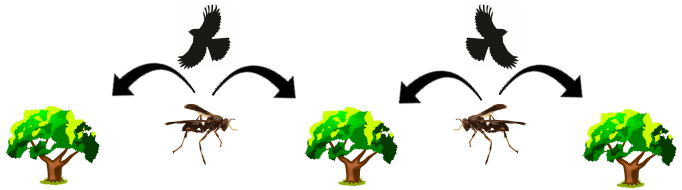

Middle population traits


Southern population traits

In our example here, we have seen that across three groups of Diploglottis campbellii, that some private gene versions exist in each group and that all of these are valuable. If any group becomes inbred to the point of failure or is lost for whatever reason, its special traits will be lost too. If for example, viral resistance from the middle group makes it into 15% of the total population, they may be the only survivors of a new wave of viral spread in the climate change future. Genetic diversity is adaptability, in itself an intelligence and a solution to challenges.

3. Seed collection case study IV

3. Flow of genes over the landscape: We may look at the distribution of collection sites over the range pictured for Small Leaved Tamarind and think this is a long distance, however in genetic terms common sense is misplaced. Our species, distributed over the range of 100 kms would have been very recently connected, as we know the regions of NNSW and SEQ were once largely connected and contiguous rainforest, just 150 years ago. We also know that insect pollinators may travel a few kilometres between plants on a single day and seed distributors for its plant family (Sapindales), can travel hundreds of kilometres in a single night. As such, genetic differences that would result in the common term ‘local provenance’ are not likely in this range. Secondarily, the assumption that local genetic differences would have occured in such similar habitat and range is very unlikely. This is due to the fact that genetic changes that affect actual changes in important regions of the DNA of organisms are unlikely and take several thousand years to occur naturally. During such a time frame, seed would’ve been passed back and forwards from all ends of the habitat range of 100 kms, many hundreds or thousands of times. It is more important by far to avoid inbreeding problems in rare species that are low in number. In low numbers and fragmentation, it is likely that the gene pool has been broken up across human disruption in the landscape that interrupt their pollinator or seed distributor travel because they are too large or inhibitive (farming plots may contain chemicals that kill pollinators for example) in suitable habitat for the creatures that pollinate and disperse them.



Pollen and dispersal keeps a species genetically connected

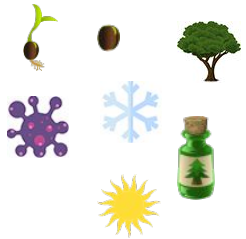
In the fragmented environment isolated groups are forced to inbreed, If there is no dispersal or pollination and there are few individuals, there are no options left. Note chemical based farming between fragments might kill and discourage pollinator travel.

3. Seed collection case study V

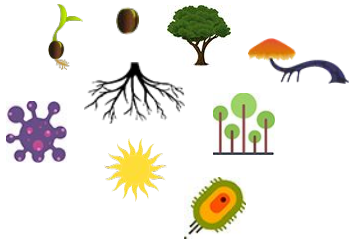
4. Growing big and mutating: Our species example, the Small Leaved Tamarind is recovering from being very low in number. In genetics, we assume that from 104 trees surviving that in actual genetic terms, there are maybe the equivalent of 15-20 genetic types in that 104 trees. Example : Think of 'race' in humans; Asian, Arabic, Indian, European, etc.. We are able to roughly identify where someone comes from, because even from a group of millions of individuals, there are strong similarities that mark a group. These genetic markers that are similar in a group are literally aspects in that group's DNA that are low in diversity and thus we can recognise peoples by these. It proves that even when there are differences, there are always a great many similarities in a species (104 survivors aren't going to be all completely different from each other)... Now if you take a group from a single race, from a single town and disallow breeding with other towns, you will see inbreeding much faster than if three towns intermarried. That is the short term issue of inbreeding and fragmentation. But what's the long term solution?

SOLUTION: Imagine here that our species has a gene pool containing the example traits on the left, we've ensured we're avoiding inbreeding short term, now we need to see NEW gene versions arise. This relies on accident. Genetic errors create new gene versions. Imagine if each single plant had a 1 in a million chance of winning the genetic lottery and having offspring with a new colour of fruit for example, now give every tree that exact same lottery chance. The solution to low genetic diversity is to avoid further inbreeding, maintain breeding opportunities for the whole gene pool and increasing population size. Over time, a few of the trees will create new mutations in their offspring and expand the future potential of the species.

Gene Pool in the year 2004 (104 trees)



Gene Pool in the year 2100 (65,000 trees)



OUTCOME: Here our species was low in diversity when it narrowly survived in extinction at the turn of the 21st century.
With good breeding, large population size and time, new genetic sequences will appear (examples include, new leaf types, variants in root growth or leaf shape, longer limbed or different growth habitat variants, bacteria resistance or new fungal symbiosis, and new flower colours to attract new pollinators).
Realistically, it may take some species thousands of generations to fully recover from low genetic diversity.

Note lost genes: One critical reason we must think of genetic diversity in these terms is the harshness of change. The natural world has eliminated virtually 99.9% of all species that previously existed on the Earth. Droughts, fire, continental shifts, introduction of competition, predation or disease, etc. In our imaginary example above, frost tolerance and antifungal extract was lost in the future. Why? Because at any stage, any portion of a species could be lost to any force listed above or other. We must expect species to lose genetic diversity through events. Their best chances to survive and offer a new version of themselves to the future is to allow species the diversity and the numbers they need to mutate new ways of being. Note that most of what lives on the Earth today is always becoming more and more unlike its ancestors, always changing and adapting. Saving species from extinction is not solely about single species, it's about protecting the radiance of the tree of life, so that what we preserve now can continue to grow and change with the Earth and move in new directions.

3. Seed collection case study VI

5. Avoiding genetic loss from a single group: Natural events such as those listed in point 4 are often out of our control. No matter what we do to save our species, there will be threats and losses in its future. Focusing on the near term (10-100 years), during our time, we must ensure that endangered species living in fragmentation, lose as few of their genes as possible (particularly genes that have only survived in one location). This is one critical reason to collect widely and ensure nothing is missed. New mutations will occur if a species continues to exist, but perhaps, it will never again evolve frost tolerance or drought resistance, evolutionary chances for specific positive opportunities are low. Example of privately held genes below (We know of 20 sites in NSW and 5 in QLD where our species exists). For ease of explanation, let's refer to our example of having just three groups (Northern, Middle and Southern).

(Imagined Traits)


Healthy growth habit


Anti-fungal extract


High germination rate


Drought resistance

Northern population traits

Middle population traits

Southern population traits

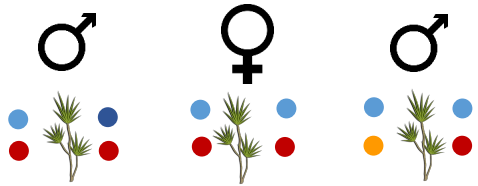
OUTCOME: Assume that breeding programs for the species continued in isolation, and wore the effects of inbreeding depression, collecting only within 20-30 kilometres. The Southern population is destroyed and, it was the only group that contained the genetic sequence for drought tolerance. In a changing world, this may have been the only variant of the species that could survive beyond 2100. Although hypothetical, this scenario highlights clearly the risk of incomplete collections and breeding for endangered species.

6. Avoid flooding the gene pool with poor material: All of us working with endangered species are fighting to preserve species with a low chance of long term survival. This is due to the changing Earth, ever encroaching human impact, chance of losing small pockets of survivors to events and genetic deterioration and other threats. Under such circumstances, the only viable approach to those passionately committed to the endangered species of the world is to take every step to avoid worsening the situation by poor collection. Imagine that from those 104 surviving Small Leaved Tamarind, that we only collect 30% of their genetic diversity. We take that and grow on thousands of trees and repopulate the landscape with low diversity. For the first generation, they may even seem okay. But inbreeding accrues over multiple generations and pollinators do not select the most diverse trees from the forest, only what they sense and find. By planting out low diversity trees we risk flooding the pollen and dispersal mechanisms of the species with low diversity material. This will become dominant in the gene pool of the species and greatly outnumber those small groups of survivors (carrying the other 70% of the gene pool). Unfortunately, poorly conceived expansion programs for endangered species can be the final straw that sends them to extinction.

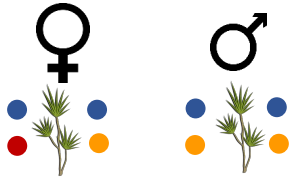
3. Crossing - Recessive genes

We know that fragmentation, low genetic diversity, inbreeding depression and lack or loss of pollinators or seed dispersers all threaten endangered species. But can inbreeding depression be reversed? Crossing from the most genetically different survivors of your species can help. From Page 14, we showed how a species of palm that are becoming extinct because they were inbreeding and suffered from both bacteria predation and poor germination due to genetic faults. Rather than waiting for inbreeding to kill the species, we have captured seed from specimens from a second patch of survivors 30 kilometres away. They contain genetic differences and both populations have lowered inbreeding depression (bad results of inbreeding) by being crossed.

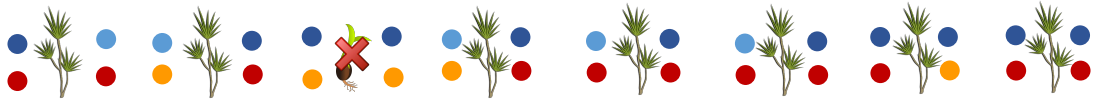
- **GENE 1** Dominant - anti bacterial resistance gene
- **GENE 1** Recessive - weak anti bacterial resistance gene 75% chance of being predated on before breeding
- **GENE 2** Dominant – healthy germination gene
- **GENE 2** Recessive – poor germination gene



Population 1 is riddled with weak bacterial resistance genes and most are predated before they can breed (one copy of the poor germination gene is present in one male, but is inactive and thus unknown).



Population 2 is carries mostly poor germination genes and suffers from very low germination survival rate. It has only healthy copies of the bacterial resistance gene.



Now select random a male and female to breed from each from group, passing on half their genes.
3 out of 5 of our parent generation had an inbreeding issue. From random breeding, only 1 of 8 of the crossed offspring has reduced fitness and fails to germinate successfully.

This approach has been taken with several successful genetic rescue projects around the world. However, it does come with it's own specific risk. What if there are two or three populations of your endangered species and one of them is actually an yet unknown, different species? We explore this on the next page.

3. Crossing for genetic rescue - hybridization

For ease of example, we'll use an imaginary animal species (many of the same rules apply), *Papillon rosa*. This butterfly has a reddish appearance. This is produced by genes that controls the amount of the red melanin colouration produced (just like human skin or hair). These butterflies also grow larger due to a gene sequence for size. They have high food supply in their natural habitat at the base of waterfalls, rivers and creeks so this size of 12cms is sustainable.

Unknown to researchers, they produce a high volume of an antifungal molecule that protects them from their wet environment

They are endangered due to deforestation in their lowland wet habitat.

Papillon rosa
(12 cm wingspan)

River and creek habitat

Papillon hybrid
(9 cm wingspan)

Papillon xanthos
(6 cm wingspan)

Cliffside habitat

Papillon xanthos diverged 100 000 years ago from the waterfall species and ventured up into the mountains, they are yellow because their genes regulating for colouration have changed slightly, producing far less red pigment (red dye when weak looks yellowish). They are genetically different enough to be a different species, but are still from the same genus.

If they cross breed with the P.rosa their offspring are hybrids between the two and don't belong to either species.

***P.xanthos are very small as they've evolved to a low calorie environment of cliffside flowers**. *
P. Xanthos are endangered due to excessive harvesting of their mountain food plants for human use.

Conservationists decide to cross P.rosa and P.Xanthos. Highland and lowland specimens are crossed to add genetic material from genetically similar species and increase the gene pools of both.

The resultant hybrid is a blend of the two colours, this alone does not affect their survivability. However, P.hybrid cannot survive well from the low calorie diet of the mountains because it is too large and they suffer a 76% mortality rate before breeding. P.hybrid also produce a lower rate of the antifungal molecule than P.rosa and suffer an 67% mortality rate in the lowland areas due to fungal infection. In this example, a great deal of breeding opportunity was wasted and although inbreeding was reduced by crossing, fitness was lost as a consequence. **Hybridization does not always go badly** and actually has a considerable place in evolution. **It can in fact create fitter offspring than their parents from either species**. It is a risk to undertake if due diligence and research have been followed.

3. Crossing - Biogeographic Barriers I

How do they apply in the context of limited gene pools

In this example, a species is found on both sides of a biogeographical barrier but for unknown reasons, seed is no longer distributed around or over it and may not have been for 100 or 1,000 years. Normally, this would constitute a situation where local provenance thinking would suffice, the groups are seperate, no need to interfere. However, in this instance, forest A has been severely logged and is now losing some of its genetic diversity.

Let's call this species ***Arbor tritone***. There are there three genetic variants in the population. Dark green, light green and yellow.
The degraded group on the left only have one tree left of the yellow type. This represents the last tree carrying the yellow gene in the cleared forest. It could easily be lost by random events; virus, drought, cyclone, fire, etc.

A

Bird distributor : local seed distributed only

Mountains not crossed by birds

B

Bird distributor : local seed distributed only

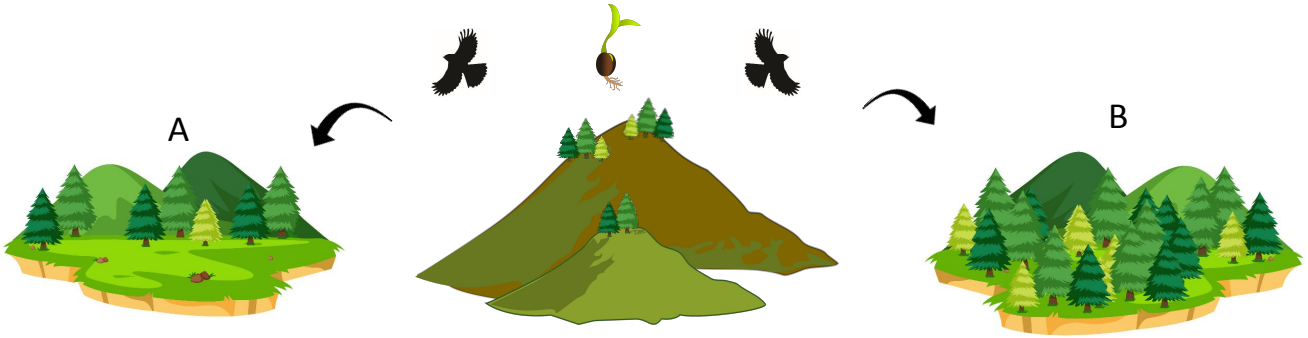
In such a scenario, we must ask if this boundary is truly interrupting genes from crossing the mountain within genetic time frames. A series of good long surveys will demonstrate that species are sometimes found in unexpected places, even when massive barriers exist. If we consider A as properly and completely separate from B, we may miss an opportunity to avoid its genetic degradation. How do we know that in the past the barrier didn't allow pollen or seed flow? What may have changed? Perhaps a disperser went extinct? Perhaps the forest used to grow all the way around the mountain? **How did the species come to exist on both sides?** Are the microclimates similar on both sides? A human damaged world, may at times need human intervention to repair it if the changes are too great. If population A on the left dies out, it dies out. Our role in such scenarios is to consider the greater benefit and the best outcome for our species.

3. Crossing - Biogeographic Barriers II

How do they apply in the context of limited gene pools

In this second example the biogeographical boundary was less severe in scale than the first. Field studies, hiking and exploring discovered that indeed some surviving individuals from our species were found on the range between both populations and bird dispersal was found to be transferring a small amount of seed from the range to both populations A and B.

In such an instance, we would again imagine that a cline exists, where from the far west of the range of A to the far east of the range of B we would expect to find a gradient of genetic material, becoming more and more different from one end to the other. We want to preserve that as best we can while avoiding inbreeding.



In this scenario one has to consider what percentage of seed to collect from each area. That will depend on the following.

A) How inbred is your local **group A** already? If there are only 100 or 200 individuals left, follow all our other rules for seed collection and also limit your total seed collection to a maximum of 50% from **A**.

B) Can you collect from specimens on the **barrier**? Are the locations of trees known? If you can, collect 25% from the **barrier**.

C) How diverse is the population **B**? If they are healthy and diverse, consider how far your seed collection will be from population **A** in kms. Refer back to pages 22, 23, 24, 30 while you consider this. You might decide to collect perhaps a further 25% of your total seed from **B**.

D) If **A** is severely degraded with only 10 or 20 survivors and you see no major reasons NOT to collect solely from the **barrier** and from **B** consider a complete genetic transplant, collecting only from outside of **A** for the purpose of genetic rescue. Bring in some new genetic material, save your species.

3. Crossing - complex ecology

In this example we have two Endangered Species, the **vine** and the **tree**.
At the highest altitude, both exhibit smaller, more contained growth habits.

The vine contains a cold tolerance gene that is more common at 600-1,200 meters, where it offers selection advantage in colder years and at increasing altitude. This selection has reduced the gene pool at higher altitudes as vines that don't carry the cold resistance gene die out and take their other genetic diversity with them. The vine is now disappearing at 1,200m and 600m due to inbreeding and lack of dispersal, new seed is not popping up and germinating. Historically, seed has been provided to the mountain top by birds nesting at 600-1,200 meters, this was the seed source. Poaching and loss of tree habitat for nesting reduced bird populations dramatically, thus there is only a small seed source for high altitude vines. The solution lies in restoring bird populations to recover the vine at high altitude and its genetic diversity.

The tree has become endangered due to logging, the survivors are in small disconnected clumps at all three altitudes. The surviving trees are too far between, for their natural pollinator wasp to travel between the disjunct groups. Also, the tree displays no genetic differences by altitude as the vine does, ilts growth pattern difference in each altitude is solely affected by the low nutrient at higher altitudes and cold damage that naturally prunes projecting branches. Thus, collection from seed at different altitudes, propagating and planting would be acceptable to boost diversity and prevent extirpation (local extinction) at all altitudes.

The final solution would be to plant trees within the broken range of the pollinators between survivor trees at all altitudes to reinstate pollen connection. Over the longer term this will recreate the nesting habitat for birds that distribute the vine seeds. In the short term; manual collection, propagation and distribution of vine and tree seedlings and installation of nesting boxes for birds are necessary until the ecological relationships are restored.

Vine	**Tree**	Presence of bird nesting and pollinators at each altitude	**Habitat**	
				Mountaintop 1200 meters
Alpine variety – small rounded herbaceous (85% carry cold tolerance gene)	Alpine variety low scrubby habit			
				Cliffs and slopes 600 meters
Sub alpine variety – some adventitious growth (45% carry cold tolerance gene)	Sub alpine variety shrubby habit			
				200 meters Above sea Level on plateau
Lowland variety – Full length adventitious growth (20% carry cold tolerance gene)	Lowland variety Tree habit			

3. Collection across fragments I

Fragments from a former range

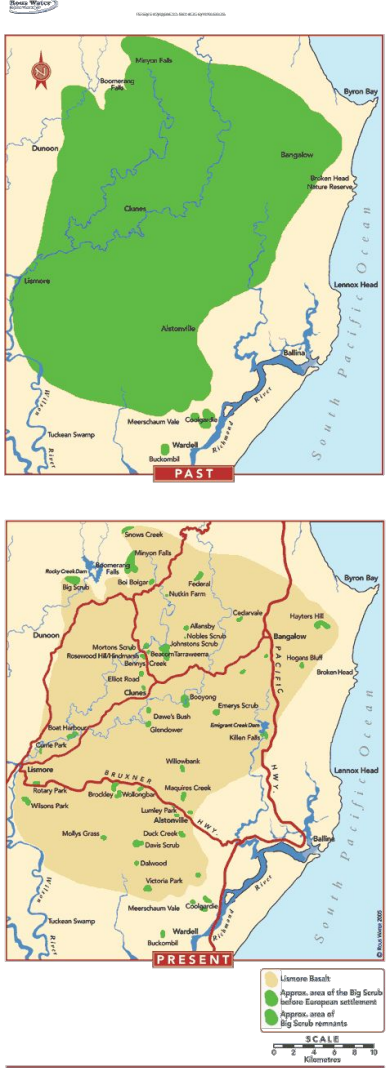
The general concept of local provenance can easily diverge from the reality of pollen dispersal, distribution and genetic time (how long it takes for changes to occur at the species level) and how those forces all interact. Understanding what was formerly contiguous ecosystem and at what ranges to call an imaginary border is always debatable and subject to new information.

In the example to the right is an ecologically discrete subunit, the Big Scrub rainforest of NNSW. Despite its range of 75, 000 hectares, it was once a completely unbroken area of rainforest. In such an instance, seed collection anywhere within that contiguous zone for an endangered species is positively argued for, as most local provenance experts would agree even across such large distances genetic connectivity would naturally be maintained over long periods of time. However. Genetic relationships don't stop over even these distances, they tend to 'cline' that is, slowly become more and more different, over hundreds of kilometres, even thousands of kilometres, not tens of kilometres.

There exists a precautionary principle for keeping tightly to 'local' seed collection; avoiding genetic pollution, outbreeding depression (collecting seed from outside the area and it performing poorly locally), accidental hybridization, or loss of a local type of a species, or even a local subspecies. Yet 'local' was a poor word to choose, as it colloquially suggests ones neighbourhood or maybe a 10 or 20 minute drive radius. It is simply the wrong word and has caused a spread of misunderstanding. 'Local' is based on; seed and pollen dispersal, genetic time for changes to occur, landscape barriers, climate and more. It is **quite a bit larger than we'd ever imagine**.

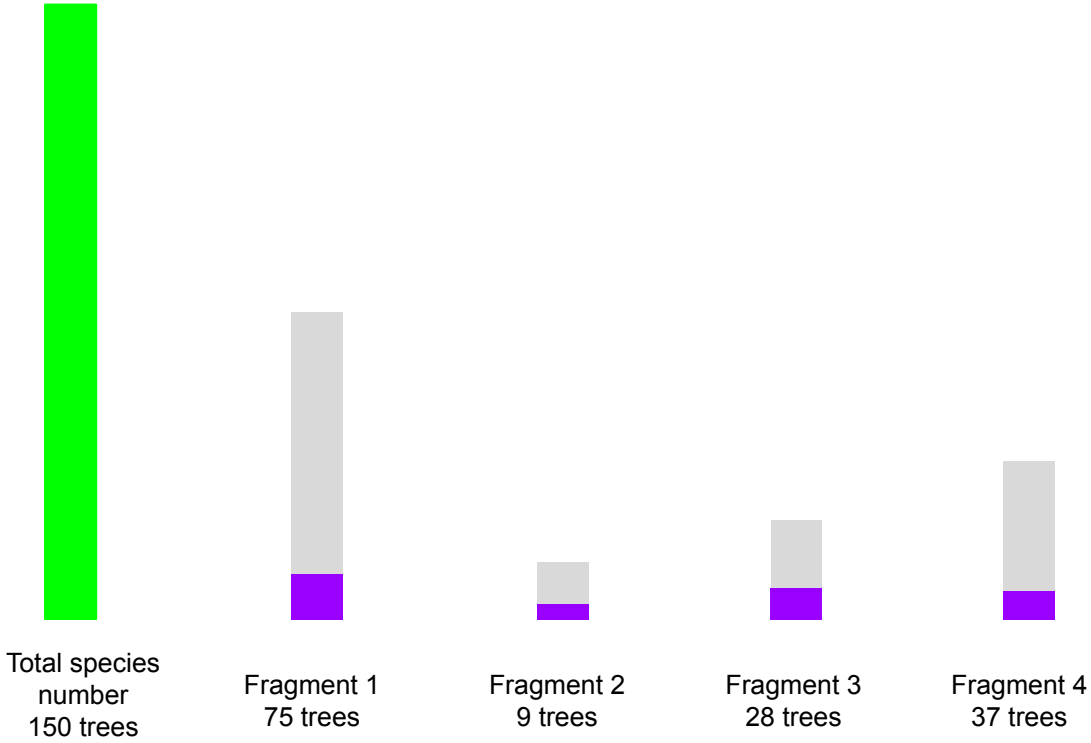
Yet for endangered species, there also exists inverse risks for not reaching out and only breeding 'locally', especially from fragments. Such as; Increasing inbreeding/inbreeding depression, increasing artificial segregation already being caused by fragmentation, risk of losing private genetic carried only by a few survivors in a small areal by natural events in local clusters such as fire.

On the next page, we will explore the risks of inaction and local collection precautions.



3. Collection across fragments II

Why does fragmentation makes things much riskier for the endangered? Because **fragmented survivors may be acting alone.** Let's imagine we have a species with 150 known survivors. They are distributed across four fragments that are far outside of their pollination and seed dispersal range.



If all four of these fragments are completely disjunct, each exists as if it was alone, as the last of its kind. Let's assume our species with 150 individual survivors was assessed by conservation guidelines and given a reasonable chance to survive the next 50 years. But we've discovered that no pollination or dispersal is happening between the four survivor locations. They are now at greater risk, they've been assessed based on 150 survivors, but genetically, they are not 150, they are isolated, smaller groups.

Fragment 1 is the largest, but being a single small group could easily be wiped out by a single event, such as; clearing, fire, drought or other event. Half the species survivors could become extinct in a single event. Fragment 2 if not reintegrated could face inbreeding depression and sudden extirpation (local extinction) because it is so small.

Regarding all fragments, the longer they sit in isolation, the greater the risks are that genetic deterioration will occur, as it is not solely single events that wipe out a whole fragment that we are worried about. It is also the risk that some individuals in any fragment will die as the last holders of genetic information not found in any other individual.

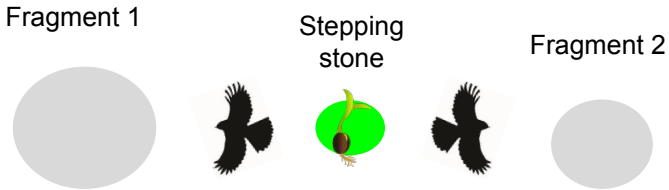
Note the purple bars. These represent that a portion of each group holds unique genes that no other group shares. All groups are valuable genetically, any lost are a loss to the species. Sample widely, integrate, don't risk losing any unique genes that have only survived in one spot to one event.

3. Collection across fragments III

Fragmented species may be acting alone

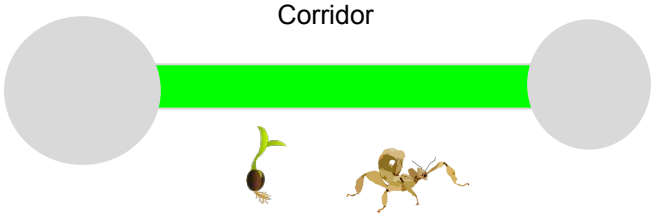
When we say that a species has 150 survivors left and is endangered, we should seriously consider its fragmentation status, ability to seed disperse and pollinate across fragments. Creation of stepping stones, nature corridors or translocating seedlings have been suggested as solutions to fragmentation issues and each have their considerations before you begin.

a) **Planting stepping stones** - we must ask how seed will be distributed and pollinated across the space between suitable habitat.



Will seed distributors use the stepping stones to distribute seed and therefore reduce the pressure on pollinators and increase dispersal? This is often a moderate costing approach.

b) **Nature corridors** - we must determine if corridors are financially feasible and if land access is going to be possible with all landholders. Also consider, how to structure the corridor to encourage its use as a dispersal and pollination vector for ecological relationships, you would want to consider habitat species for your pollinators and dispersers, as well as other foods they consume. Your species alone won't feed or house them all year! Due to funding restrictions corridors can become very thin, generally aiming for an absolute minimum of 50 metres width is suggested. Ask yourself if there is a deep centre to your corridor plans and if you can achieve this. Everyone wants a corridor, but we often fail.



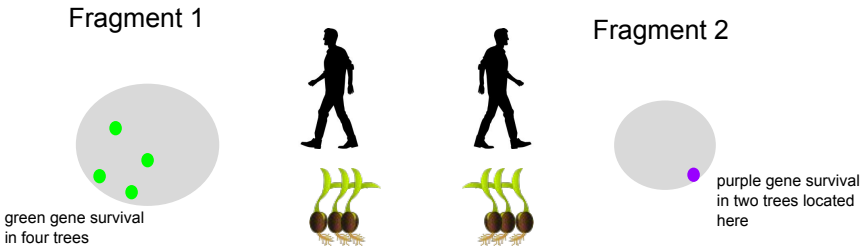
Seedling directly or encouraging pollinators?

Will sufficient funding and allied landholders/councils allow for the project to occur in a contiguous fashion? Will it reach the whole way across and be wide enough? How much time and resource can you commit to seeing this through? Corridors are often hard work, taking years, large costs and various forms of maintenance due to continued weed invasion from edges (especially when too thin), increased feral animal issues, large restoration project size, committed manpower requirements and long project duration.

3. Collection across fragments IV

Fragmented species may be acting alone cont'

c) **Translocating seedlings between fragments** - we must ask if the correct quantity and variety of seed sourcing has been used, as to best improve genetic diversity, not swamp one location with a large volume of material from another.



40 surviving plants (80 copies of genetic material)

- 50 copies of red gene
- 25 copies of blue gene
- 5 copies of green gene (private)

12 surviving plants (24 copies of genetic material)

- 13 copies of red gene
- 9 copies of blue gene
- 2 copies of purple gene (private)

In our example, humans propagate and translocate seed between these two populations. We need to consider how much genetic material is being brought across. Both groups can benefit from gaining each others private green and private purple genes (the versions of genes only present in one fragment). Fragment 1 has many more surviving plants, so we might be tempted to collect a large number from there to help recolonise fragment 2. However, in doing so we could result in fragment 2 having an even lower percentage of surviving purple gene than it does now. Purple gene makes up 1/12 of its gene pool and while we could enrich its gene pool particularly because we are adding the green gene, we could swamp out the purple gene by mass of fragment 1 material.

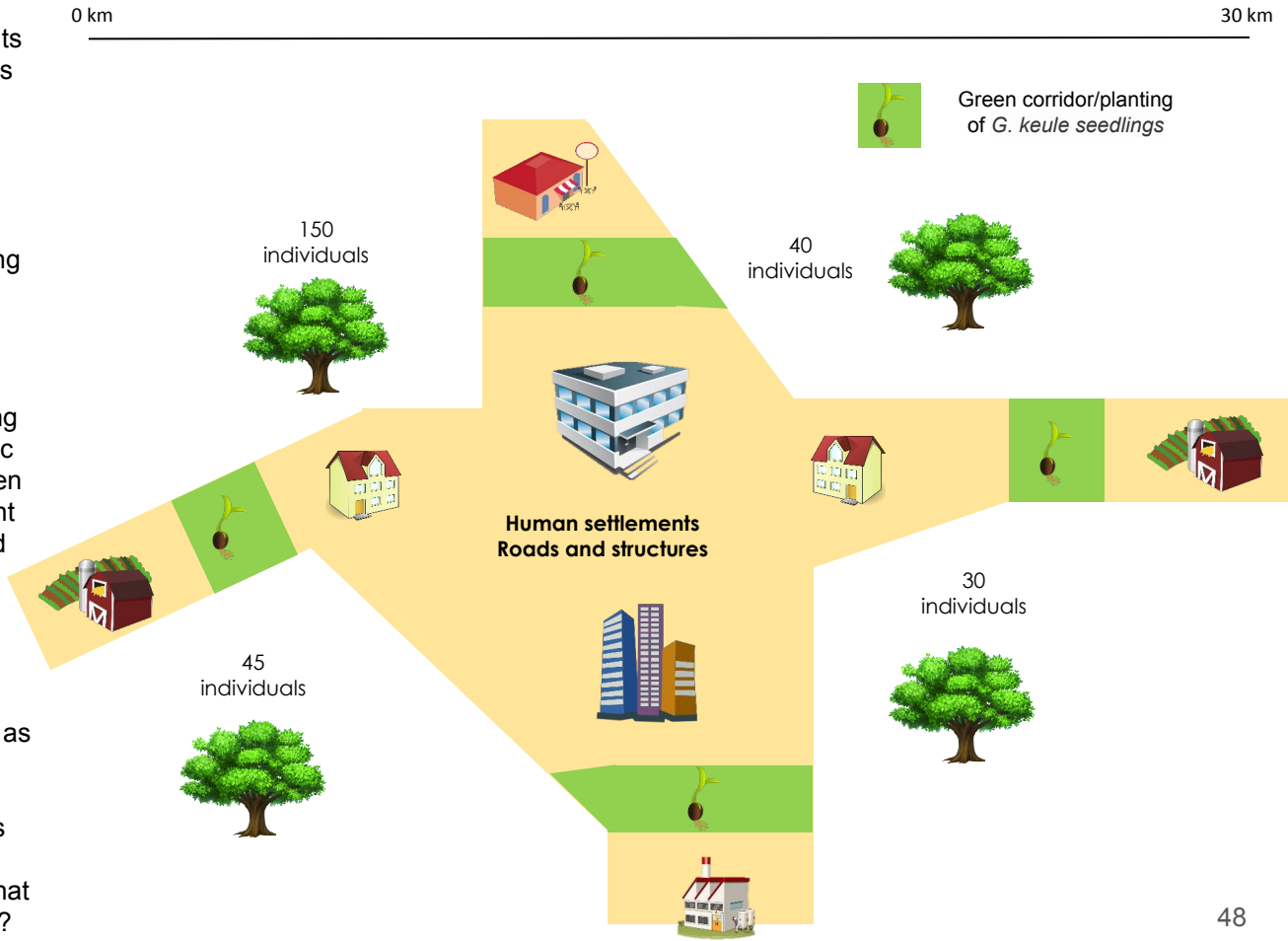
The 2 copies of the purple gene are only present as 1 of 2 copies of a gene in 2 surviving plants, on the edge of the remnant (as seen in the fragment 2 graphic). An increase in population size by translocation from fragment 1 could produce pollen closer to the rest of the surviving trees that could see the purple gene swamped out of breeding opportunity by introduced, red, blue and green genes. It only takes for a few missed breeding opportunities, a little further deforestation, or edge drying (that often kills big remnant rainforest trees in fragments) or natural death of remnant trees and this purple gene could be lost. This also refers back to wide collection methods. Leave no genetic material unsampled.

The Chilean tree *Gomortega keule*, is a highly edible, large seeded/fruited species. Its habitat has been largely cleared and disrupted by human development, including large agricultural areas in its previous range. The seed disperser for this species is thought to have become extinct and it may now only be spread by cows or rodents (native or non). Not only has the species been pushed apart with small surviving numbers per area, most surviving populations are below the minimum number suggested for short term survival against inbreeding (in this example). Less than 50 individuals is considered highly dangerous in the short term (perhaps 3 generations).

Here seed collection, propagation and transplanting of seedlings from all groups would increase genetic diversity for all. Ideally reforestation corridors (green areas) could help achieve this in a more permanent arrangement, as conservation programs come and go. First, one should ensure immediate genetic connection in the short term by translocation, then build green corridors.

In such an instance, where dispersal is poor, physical, direct green corridors are recommended as a long term solution, with the endangered species planted across these fragments to reconnect the species pollen and dispersal flow. We must always ask what fragmentation means. Across what distances can it be pollinated or distributed? By what creature and with how many other trees of its kind?

3. Fragmentation corridors



3. Parent trees I

Enough parent trees

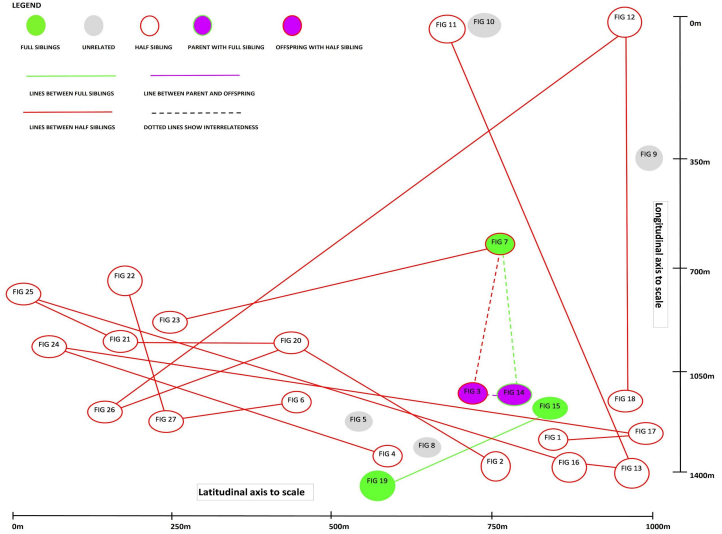
International guidelines for conservation programs invariably suggest collection from at least 20, 30, or 50 parent trees for ecological restoration, This is not being achieved by nurseries, private collectors or NGO's in many Australian ecological restoration programs. Firstly, Australians have been historically encouraged to collect from 10-20 parent trees, a figure that has become embedded in much shared Australian literature. This does not meet the standards set by any other country publishing recommendations for restoration that we have investigated.

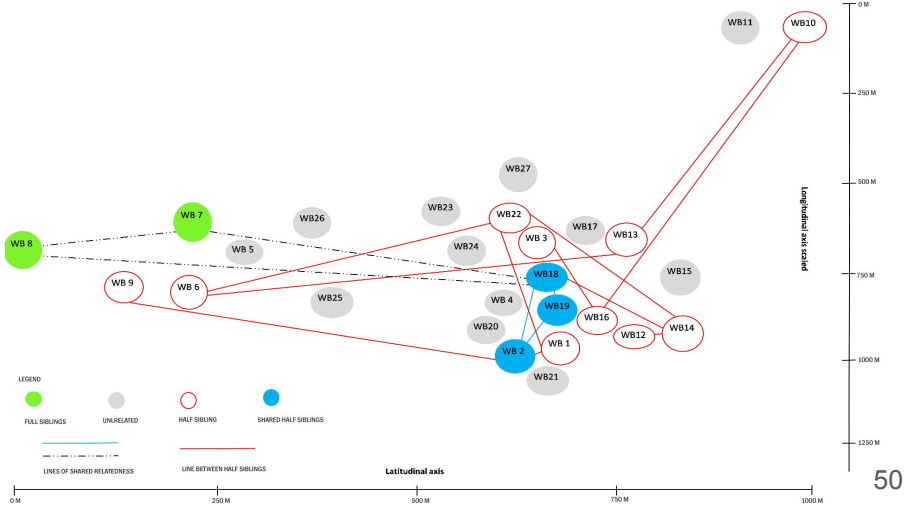
It is difficult to collect from high numbers of parent trees in ecological restoration when working with relatively common species, even harder for endangered species. Collecting from say, 30 parent trees for a species reduced to 100 known survivors requires several considerations to maximise your parent tree number as you are unlikely to know all survivor locations.

A) GPS mark all individuals as they are discovered.
B) Collaborate with others working with your species for seed.
C) Reference with Living Atlas (https://www.ala.org.au/), environment.gov.au (https://www.environment.gov.au/biodiversity/threatened/species) and other resources for known locations and expected range for endangered species, this way you can check actually GPS locations or search expected ranges by survey.
D) Check with other conservation programs or consider ordering seedlings from nurseries, determine if their sources are complementary.
E) Check literature for known historical locations for the species and perform surveys for new, unknown or unmarked specimens and locations.
F) Determine licences, permissions and access to any endangered species material before engaging in collection on private or public land.
G) Consider collecting from wildlings to increase the chances of capturing genetic material from unknown specimens.

3. Parent trees II

2. Close and far. Not only must we collect from a high number of parent trees, we should collect from both close and far individuals, even in larger areas this rule applies. In this example of relatedness by range, we would like the reader to note unexpected patterns that we discovered in genetic research of rainforest trees in the Big Scrub rainforest. In the bottom left we have F. watkinsiana (rainforest strangler fig), in this example, less than 40 mature parent trees were found in a 180 hectare area. Surprising, relatedness lines are almost always drawn ONLY between individuals that are 250 metres apart or much more. In some stunning examples, Fig 12 is only related to Fig 18, 1,100 metres away and Fig 26 over 2,100 metres away. If one had only collected from these 3 trees, far apart, thinking they were diverse, they would have sampled just 3 half siblings, despite hours of hiking between trees. Note Figs 10, 5, 8 and 9 sit near other figs in the landscape and are not related to any. While Fig 3, 14 and 7 are in close proximity and are very close family. Bottom right, A. trifoliolatum (white Booyong), a far more abundant species, we note again, an unexpected random relationship between close groups (such as the blue group - or green group) and far groups WB 10, 16 and 3 for example. Both of these real world examples show how bizarre genetic relationships really are. Always collect widely and always from 20 or more parent trees to avoid accidently capturing from only closely related individuals. Ideally, no genetic material is lost in any generation of an endangered species future.





3. Parent trees III

3. **Age of trees from ‘remnant’.** Collect from trees as old as possible, older trees are the children of the forests that existed before recent human population increases and major industrial technologies of the last few hundred years. In Australia, much deforestation only happened in the mid 1800’s, so most of the ‘remnant’ trees we see would be the first or second generation of offspring from the remnants that were left by the first wood cutters. We want the oldest possible specimens for our species as the offspring are far more likely to have inherited their genes from smaller, fragmented groups than their parents did. Repeating that, the older trees are more likely to be genetically diverse than their offspring in a fragmented, severely logged landscape.

4. **Planted, easily visible and street trees.** Thinking in terms similar to above, imagine seed was once collected from a first, second or third generation regen tree from remnant. The seed collector would have most likely taken from a few trees, or whatever was fruiting on the day. Seed collectors can’t just hike all over every remnant forever. These seeds, from few trees, who are the offspring from the true remnant trees are then taken, bred up by the thousands and we’ve already demonstrated that they’re likely to be lower in diversity than their parents, because they come from an already reduced, fragmented, remnant. Consider what fraction of your species gene pool has been destroyed by recent human activities in the last few hundred years. Therefore, we strongly recommend to limit your seed collection from easy to access trees, as they’re very likely to represent a poor genetic input for your species overall, those genes are already getting too much reproduction!

Amongst all your collections, is it always a good idea to also collect abundantly from the largest healthiest looking specimens.

3. Parent trees IV

5. **Go where the tree fellers couldn't.** Obviously it isn't practical to source all seed from 1-4 hr long hikes into rocky steep remnants, where many of the oldest remnant trees are, but we recommend it thoroughly. Areas that logs could not be removed are great places for genetic and general species diversity to have survived. These are also areas that you expect the least humans to have bothered to have sourced seed in the past. We all have limited human resources, if you find an area that is unlikely to have been sampled for seed, bring back a high volume, thousands and tens of thousands of trees are being planted in restoration projects, future seed source will need balancing out with your hard to get to, rarer types. Consider the existing gene flooding from easily accessed trees!

1c. Expanded provenance seed collection

In the case of an endangered species, an even wider collection area that would normally be regarded as acceptable (an expanded provenance) should be investigated/considered for seed collection to reduce the chance of in-breeding as a possible cause of local extinction/extinction. If there are only a few hundred survivors or less, it must be explored. An expanded provenance in your seed collection will help the species to avoid a range of in-breeding related problems and/or an inability to resist external pressures. Although at times low genetic diversity is not a cause of near term extinction, for many unlucky species, it is, and this can be difficult to detect at any point in the process of extinction. Even the healthiest looking tree may have a weakness you are not yet aware of, which may only become apparent in a drought, season bacterial/fungal growth, or other event.

Throughout this document, we have covered many reasons and examples of why bigger provenance should be considered, from genetic rescue, outcrossing to reverse inbreeding, reversing the effects of fragmentation, relatedness by range, natural and past seed or pollen distribution, biogeographical boundaries and more. We urge anyone working with their endangered species to note the colloquial and existing programs and beliefs, but to test every assumption. People are busy, and sometimes that means assumptions are respoken without testing or new information has not been integrated into common knowledge.

3. Seedlings and wildlings, why both is better (seedlings) I

Here we will summarise the benefits to collecting from parent tree seeds vs wildlings collected from the forest. We encourage our readers to do both seed and collection from wild grown seedlings.

Considerations of seed collecting

1. Predictable but limited collection period due to known fruiting and flowering times, which for some species are very short windows and may only occur once per year, or less.
2. Ease of identification by mature tree, leaves, flowers and fruits. However, very mature individuals can be very hard to ID if fruits and flowers are not present and where complex canopy, and vines are present.
3. Bigger collection is possible due to larger volumes of individual fruits than one would find in wildlings. This has pros and cons itself depending on your situation (re: when collecting from an extremely remote and remnant location bulk collection is a very good idea).
4. known location of trees and information sharing of locations. A blessing and a curse, these trees can be over harvested and dilute the gene pool with too much from the same trees year after year.
5. Parent tree is known and this gives us a vector to discover more about where the seed came from.
6. Preservation of genetic diversity at germination. Some seedlings fail in the germination phase in the wild, we know genetic diversity is lost at that stage. Collecting and germinating in a nursery might save genetic types that would otherwise be lost. This may or may not be a good thing and should be questioned not assumed.
7. Requires licence for working with endangered species however, may be possible to source seeds from your network without a licence.
8. Requires specific preparation and germination knowledge, facilities or equipment.
9. Long time to germinate, prick out, grow on and plant.
10. More difficult to prepare quickly when working with funding than wildlings.
11. Seed collection is generally a hit and miss activity, expect to go out and not get what you were looking for.
12. May need to be repotted once or twice before planting.

3. Seedlings and wildlings, why both is better (wildlings) II

1. When collecting wildlings, there may be a higher chance to collect genetically fit survivors i.e the weak and inbred may have died early in germination. However, this may or may not be a good thing if those marked unfit by death would make fit adults or succeed in easier forest starting conditions. Better not to assume too much and to collect both wildlings and seed for this reason.
2. A wildling harvest is more likely to have come from a higher number of parent trees than a similar day of collection direct from tree seed as wildlings often represent several seasons of fruiting from several trees, where seed collection is very often from only a few parent trees on a given day.
3. Year long collection is possible as wildlings are generally persistent, once locations are known collection is fairly reliable compared to seed collection, endangered seed collection days can often provide nothing.
4. Juvenile leaves look different to adult leaves in many species and can be challenging to identify. Plants from large families can look very similar at the juvenile stage also, such as myrtales or eucalyptus families.
5. Faster time from potting up, growth and planting, than seed, it might be twice the speed if done right.
6. Difficult germination or unknown germination techniques are not needed.
7. Easier to collect in high numbers if parent trees are inbred and low fruiting or are not fruiting.
8. Fragmented isolated rare trees may be infrequently pollinated and infrequently fruit, searching around the base and nearby might field wildlings from the recent past.
9. Requires licence for working with endangered species, site access arrangements, etc.
10. Private or public sites may be difficult to acquire for harvesting depending on property manager views on digging out wildlings.
11. Requires hot, wet, misting house for success and growth.
12. Can be institutionalised, small seedlings that look hardened, brown and woody might have sat at this stage for years. These are not the best to collect, they will be slow at the very least and or die at the worst.
13. Can be difficult to extract without damaging the roots, wet the soil preferably, collect no larger than 10 cms above ground size and gently lift the taproot with trowel or similar, roots attached to soil should be very delicately freed or left attached in a bucket of water.
14. Pot with fertiliser in their first and final pot, no extra handling recommended or required.

Tree ID skills of a high level may be needed to identify some species at the germination stage

3. Wildlings, how to collect them

Weather & Conditions

Where possible, collect after rain so the soil is soft, this will make extraction easier without damaging roots.

Do not collect from dry, especially dry clay soil. The roots will hold fast to the soil, be hard to remove at the nursery, suffer tears, lose root hairs and even if they look good to the eye, will make for poor growing wildlings back at the nursery. Save your time.

Size & maturity

15 cms

5 cms

As a rule of thumb, stick to wildlings with an above ground size of 5 to 15 cms. This size range only be reduced based on species, not extended! (some will be too developed at 15 cms).

Don't collect fresh germinated wildlings (usually two tiny soft leaves like a food sprout) wait a few more weeks. This stage is too fragile and the success rate will be lower for any species.

Do not collect from seedlings where the stem has hardened and browned, even though they are small. We call these 'institutionalised wildlings', they may have sat for months or years on the forest floor, they will take far longer to grow on in the nursery or may just sit with no growth. There are of course a few species exceptions to this rule.

Field tools & time

The tool for extracting wildlings is called a 'dandelion weeder', aim down at a 45 degree angle at where you imagine the bottom of the roots are. **Lift soil gently with the tool while very gently lifting the wildling with your other hand, slowly.**

Don't use bigger tools, You'll ruin the roots! Stick to dandelion weeders. This is a delicate process.

Put wildlings straight Into a bucket with 5 cms of water in the bottom and regularly wet your collection while out in the field. You can use a hand mister or hand wash them. You can wrap bundle of their roots in tissue paper.

From beginning of collecting your first wildlings to getting back and potting them up should be no longer than 2-3 hours. When you finish collection **go straight to your nursery and pot them. It's a mission not a cruise.**

Potting up

Pick the staff to do the work who you know will be the most gentle with them! **Dangle roots freely into empty pots and drop soil in around them. No prepacking the pots!** Mix a little perlite or other to make root exploration easier.

Don't stage potting with little pots and repotting later. Wildlings have been ripped from the ground, they don't need additional repotting trauma. **No forestry tubes ever!** 70mm or 90mm only!

Pot your wildlings in their first and final pot. They should stay in this one pot til they are planted. We recommend 70mm, 90mm or larger.

Wildlings must be kept warm,humid and wet once potted up, keep their leaves regularly misted, every hour. They need this for 3 months.

3. When Purchasing trees I

The nursery issue

Commercial nurseries and land care nurseries will always have limitations to their seed collection capacity, part of operating any business is that there must be a reasonable limit and attention put on any one expenditure. A general issue persists in the sourcing of native plants for nurseries. Whether the nurseries are owned by individuals or NGO's such as landcare groups, there always exists limitations in time and money spent in acquiring seed. Nurserymen must either forgo time working in the nursery and go seed collecting themselves or pay external contractors or volunteers to collect seed. As such a tendency exists to collect a large amount of seed from a small number of individual trees (limited genetic material collected). The higher the number of species a nursery works with, the higher the limitation on genetically diverse collection. International guidelines for conservation programs invariably suggest collection from at least 20, 30, or 50 parent trees for ecological restoration which is not being achieved by nurseries, private collectors or NGO's.

The impossible expectation on seed collectors: if a seed collector follows the general international recommendations of collecting from 30 parent tree for each species and collects seed 5 days a week, 260 days a year, 300 species, they would need to find 9,000 trees per year to collect seed from, approximately 34 diverse trees per work day. This task is not possible by any single seed collector, nursery, or landcare group. Instead, it is likely to require very large teams to do completely. This is why groups need to work together to increase the genetic diversity of seeds.

When seeds are collected, they are usually collected from easier to reach trees which are often younger and lower in genetic diversity . This leaves nurseries with seeds that are collected from too few, and young, parent trees.

3. When Purchasing trees II

Purchasing from several nurseries

Purchasing seed for endangered species instead of collecting oneself requires a simple, but new approach to purchasing. We are recommending that you spread your purchase of the number of trees needed, across multiple nurseries within your suitable zone.

ReForest Now's own genetic analysis of rainforest tree nurseries in NNSW demonstrates that those may collect from just 4-8 parent trees and collect bulk seed of those trees. Given the 300 species they work with, this still represents collection from around 2,100 trees per year, which represents enormous on the ground effort for small crews of seed collectors. We all need more help with seed collection.

No# of parent trees from cohorts of trees from each nursery in NNSW

Nursery	*A. trifoliolatum*	*F. watkinsiana*	*D. australis*
A	4	7	8
B	5	7	4
C	7	6	NA

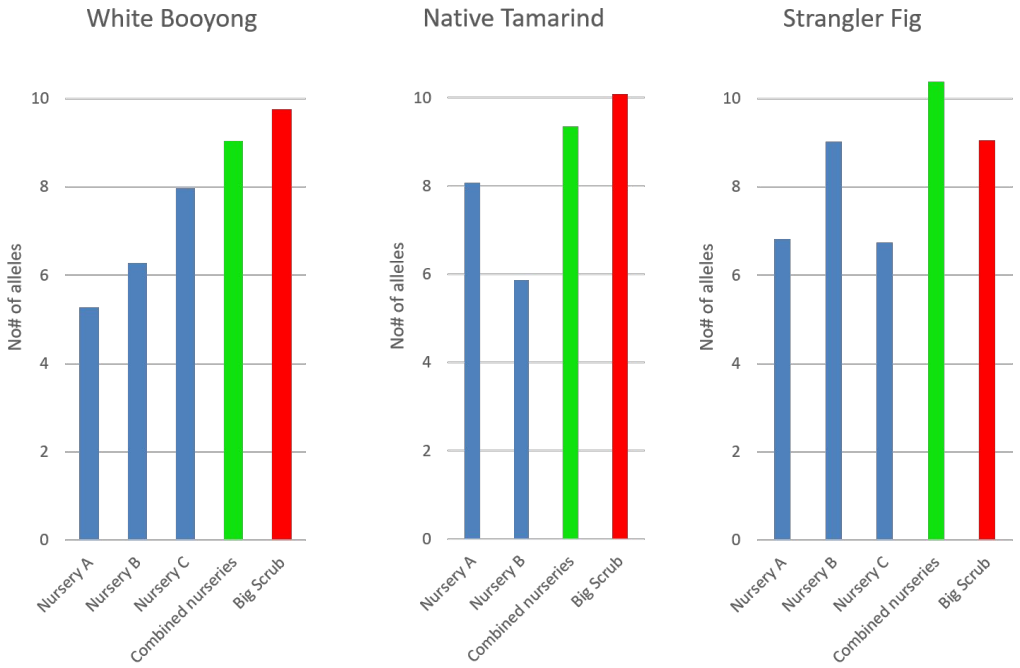
Tree planters are working at greater scales than ever before. Please don't contribute to gene swamping by planting thousands of plants from the same few parent trees!

Each of these nurseries have their own seed collectors, locations known to them and localities for collection. These relatively small numbers of 4-8 parent trees per nursery can be improved by combination of all of that work by those separate crews.
Rather than assuming that mixing seed from different nurseries will increase genetic diversity, we will demonstrate it factually on the next page.

3. When Purchasing trees III

Purchasing for several nurseries, boosted genetic diversity

Here we demonstrate collection of three different species, from just one single rainforest remnant (red bars). Conversely, rainforest seed collectors working at all three nurseries (blue bars) would not be constrained to collect from a single location and therefore more likely to capture a variety of genes only present in a single location (this would boost their results seen here). Therefore, note that despite seed collectors capturing genes that are very likely from a variety of remnants, one collection from remnant trees of 35-55 metres yields higher diversity in every example (red vs blue).



The green bars represent the combination of seedlings from each of the nurseries. This is far more an appropriate level of genetic diversity to match what we found in old remnant trees. This is a working solution that is in reach. Collection from multiple nurseries can boost diversity.

Note in the example of Strangler Fig, that combination (green bar) yielded higher diversity than the remnant. This was due to the fact that seed from that species is collected long range (definitely not local provenance, not even regional provenance). This is also evidence that genetic diversity can be boosted by long range collections.

Summary: Creating the future

Every species on this planet tells a story, a record of survival, evolution, trial and tribulation written in genetic code recording how it came to where we find it today. These stories are forged by change: in genetic composition, opportunity, distribution, and the consequences of endemic threat and loss. At this critical crossroads we have a choice, as conservators of our planets environmental integrity. We can stand still and grieve the monumental loss of species threat and loss have wrought, or we can embrace the opportunity reinvigorate and restore our species so that they can not only survive but continue to change, to speciate.. Speciation can create new environmental adaptability, and propagation of species which will outlive our lifetimes and those of many who follow. But this requires safeguarding genetic diversity to ensure the new floral vanguard can withstand the challenges to come. Change and adaptation are hard fact of life, but with the right support mechanisms in place you are not just protecting your chosen species, but ensuring their adaptability now and into the future. Conservation genetics is essentially about finding the ways and means to protect the Tree of Life. When a branch falls, our environments radiant composition loses another critical element. Each branch nurtured and sustained has the potential to grow, adapt, and evolve, expanding our environmental integrity immeasurably.

Long term survival requires what Darwin refers to as 'deep time' and devoted stewardship. This can be meaningfully supplemented by encouraging new mutations, wider distribution, and broadening populations. Invariably, our planet will renew and regenerate in accordance with its own plan, irrespective of the cataclysmic environmental crises currently at play. But if we can facilitate and support ideal outcomes for endangered species in our individual lifetimes, then that one small step by man CAN represent a giant leap for mankind, and our environment.

Thank you for reading and please feel free to contact us for further information at info@reforestnow.org.au

Thank you for your time.

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