

ENDANGERED SPECIES PROJECT REPORT 1/2



Fruits of the Small Leaved Tamarind (*Diploglottis campbellii*)

Objective 1 Delivered
by ReForest Now



Fully funded by the
Wettenhall
Environment
Trust
(\$5,000.00)



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

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

Objective 2: Produce a PDF resource that describes to people working with endangered species (not of a scientific background), practical guidelines to collection, propagation and planting to maximise genetic fitness and likelihood of survival. *Underway*

Produced by Maximo Bottaro cert III NAR, BSC, BCLIN, Hons 1st (rainforest genetics) edited by Zia Flook Bsc, BA, M.S. Candidate on behalf on ReForest Now

Thank you to ReForest Now volunteers; Michael Martin, Tashi Maizey, Elise Landry, Zachariah Holt, Johannes Beer, Sahar Shimon, Miriam Ter Borg, Odin Runga-Covington, James Skalkos, Aya Trumpet and Andy Bowles for helping to collect, propagate and care for our Small Leaved Tamarind trees.

Example Species - Small Leaved Tamarind

The Small Leaved Tamarind acted as our subject for demonstration for 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 & 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.

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 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. The species germinates rapidly and is then depleted 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. Many of the rainforest trees we call remnant trees, are in fact first generation recolonisers and are less than 150 years old, others may have been better off due to longer rests in the seed bank.

(Right) A *Diploglottis campbellii* AKA Small Leaved Tamarind. Mature leaves (quite different to juvenile) can be seen here alongside ripe three lobed 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. Studying and working with such a rare species, several uncertainties exist and little literature exists on the tree.



(Above) Juvenile leaves of *D.Campbellii*.

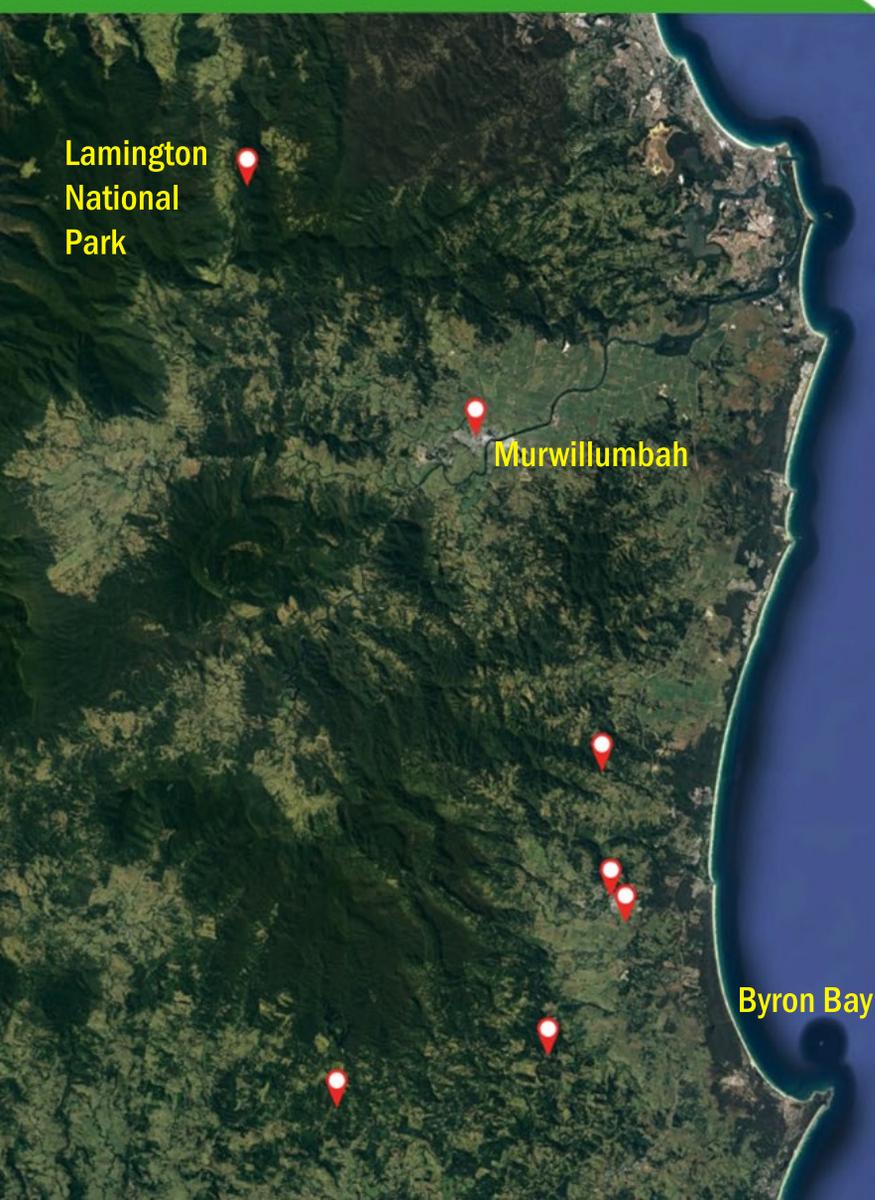


Collection Map - Searching for Diversity



(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 an area of 1020 square kilometres to ensure 'a wide net' approach to genetic sampling.

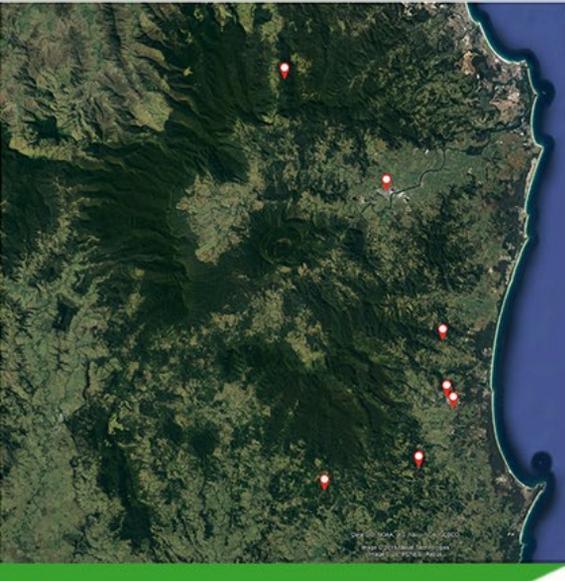


(Bottom) Fragmented plant populations are left with the risk of being unable to share genetic material with close enough members of their own species, as insect pollinators can travel only so far between forest patches and sometimes rely on pheromonal cues to guide that may not be sensed over such long 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.

The genetic benefits of wide range collection



Referring to the collection radius of 1020 square kilometres and 100 kilometres from furthest specimens 9 (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 large and genetically healthy meta-population that can survive and mutate to create new variants for the future.
5. To reduce the risk of losing a local population entirely through a single threat (fire, drought, etc).
6. Avoid flooding the gene pool with low diversity, high volume plants.

BENEFITS TO THIS APPROACH

1. Capture of 'Private genes': Referring to the map, 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 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>

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

POSSIBLE SPECIES TRAITS

-  Healthy growth habit
-  Antifungal extract
-  High germination rate
-  Frost tolerance
-  Drought resistance
-  Viral resistance

Northern population traits



Middle population traits



Southern population traits



In our imaginary example here, we have seen that across three groups of *Diploglottis campbellii*, that some private alleles exist in each group and that all of these are valuable. If one group inbreeds or is lost for whatever reason, it's 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.

'wide range collection continued 1

BENEFITS TO THIS APPROACH

2. Reduction in inbreeding: Reports indicate that the species suffer from inbreeding, via weak growth in some specimens and failure to produce fruits. Simply, the root cause is likely the presence of 'recessive genetic sequences'. This simply means for example, that in the DNA of Small Leaved Tamarinds that can't produce seed there exists only one version of a gene that codes for fruiting and that code is not functioning.

To visualise it,

Many species have two copies of every gene. If one copy is a recessive and not effective, it won't affect the plant, as long as it has a dominant healthy gene as well.

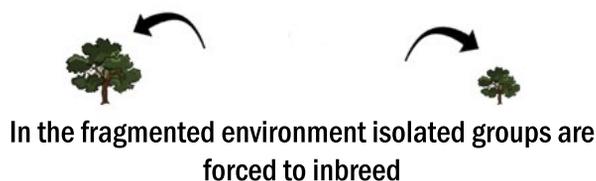
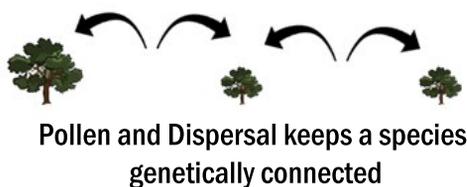
PLANT GENES



OUTCOME: Here 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 fruits very poorly/not at all. Plant three carries one copy of each gene, but because the healthy germination gene is dominant is fruits just as well as Plant 1.

EXPLANATION: Crossing surviving plants together reduces the chance that the weak germination gene can become too fixed in the group. Think of it like having a large handful of MnM's. You have a lot of different versions of them in your hands. Now imagine taking just a small handful (having an endangered species low in number) and getting three red ones and one blue. If that red MnM is a recessive gene it is easy for your group to become unhealthy, we call this inbreeding depression. In a large group with lots of genetic versions it's far less likely to get two copies of the same recessive gene and have an issue, it is normal for a species to carry recessive genes that don't function well, but these don't affect the wellbeing of the group usually. In some species, they can exist for a long time with genetic low diversity and have no serious genetic problems, but it is a big risk to assume this and there are many other risks associated with low diversity.

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 NSW 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. Secondly, the assumption that local genetic differences would have occurred 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. More important by far, to avoid inbreeding problems in rare species that are low in number, where it is far too likely that the gene pool has been fragmented as habitats are far apart across roads, paddocks and large breaks in suitable habitat for the creatures that pollinate and disperse them.



'wide range collection continued 2

BENEFITS TO THIS APPROACH

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, 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. 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?

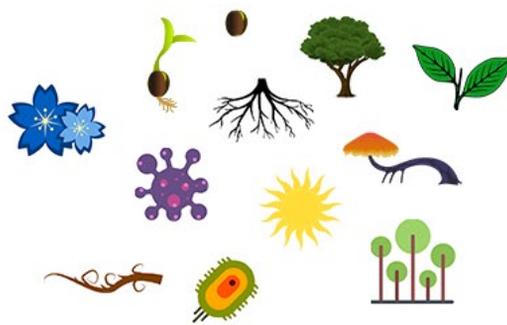
SOLUTION:

Imagine here that our species has a gene pool containing the example traits we mentioned earlier, we've ensured we're avoiding inbreeding short term, now we need to see NEW genes 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 diversity is to avoid inbreeding, while 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).

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, 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 unlike it's ancestors, just a few million year agos. 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.

'wide range collection continued 3

BENEFITS TO THIS APPROACH

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, challenges and losses in its future. Focusing on the near term (10-100 years), during our time, we must ensure that endangered species do not lose any of those private genes we mentioned (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: 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).

Northern population traits



Middle population traits



Southern population traits



Climate change induced wildfires in 2025
destroy entire southern population

OUTCOME:

Assuming that breeding programs for the species continued in isolation, wearing the affects of inbreeding depression and collecting only within 20-30 kilometres, an entirely possible scenario occurs. A whole region loses its surviving Small Leaved Tamarinds, 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. 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.

This is the one message we would hope to share; for those of us working with endangered species we must be vigilant and conscious of their delicate gene pools, seek to source appropriate material and reconnect those valuable differences that wait in the corner of a little walked valley or further from home than you might expect one should travel for seed collections.

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Maximo Bottaro ReForest Now Coufounder