Florabank Guidelines

Module 5

Seed Sourcing

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Key points

Determining where seed should be sourced is amongst the primary decisions for ecological restoration projects.

The location of a seed collection is known as its provenance. Seeds can be collected from a geographic location close to the restoration site (local provenance) or from a more distant geographic location (non-local provenance).

Local provenancing has been widely adopted, but alternative strategies address concerns associated with habitat fragmentation, maintaining or maximising genetic diversity, and climate change.

The choice of provenance strategy depends on many factors, but the overarching objective is to pursue a seed-sourcing strategy that delivers short- and long-term success in a changing environment.

A generalised decision tree is provided for selecting a potential provenance strategy given the state of the local provenance and the site being restored.

There are likely to be restoration benefits from capturing greater genetic diversity in seed collections by sourcing seed from as many well-spaced plants across a population as is practicable. Numbers and locations of seed sources will depend on the provenancing strategy for that species.

Keep seed collections of different provenances separate and record the source details of each collection to: help facilitate selection of provenances once a provenancing strategy has been chosen, and provide source material for embedding experiments within restoration activities to understand the costs and benefits of alternative seed sourcing strategies.

Introduction

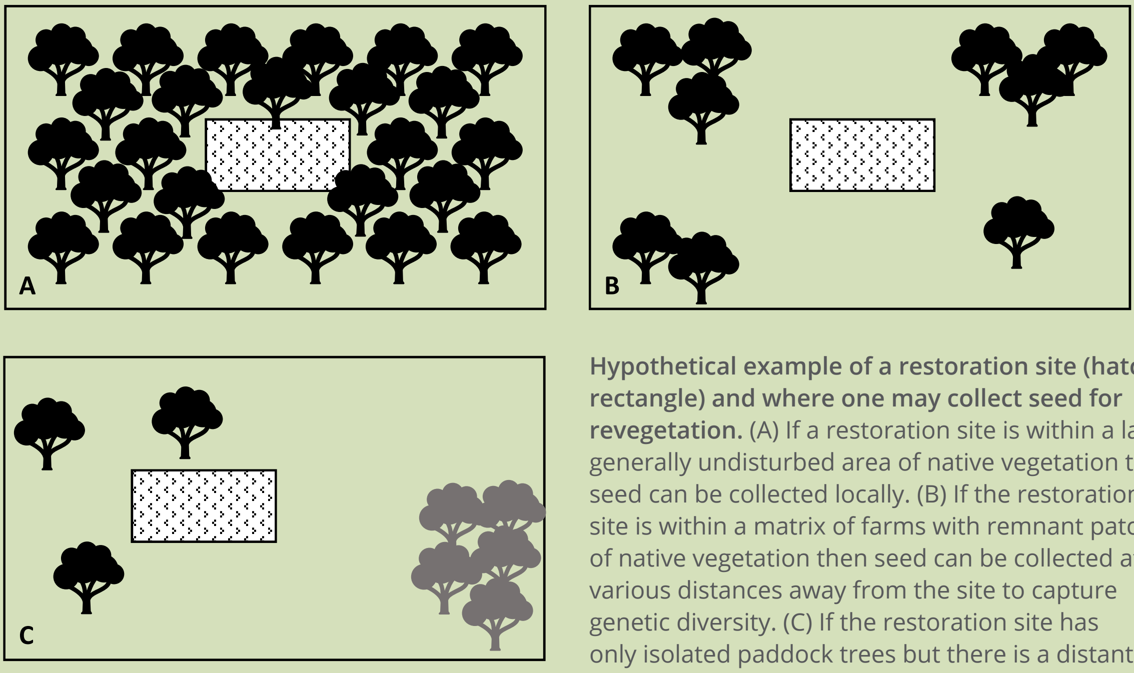
The extent of modern-day human disturbance on natural ecosystems is vast, and evident across aquatic and terrestrial biomes. It has been estimated that over 2-6 billion hectares of terrestrial ecosystems have been degraded or damaged through land-use and climate change (Gibbs and Salmon 2015). These disturbances are contributing to the loss of biodiversity at a pace exceeding background extinction rates, heralding the 6th mass extinction (Ceballos et al. 2015). Restoring ecosystem functions and services is a key mitigating pathway to stem the loss of biodiversity. Where available, a reference ecosystem is often used as a benchmark to guide and monitor the recovery and adaptive management of the restoration activity (Gann et al. 2019; Camarretta et al. 2019; Module 1 – Introduction). A reference ecosystem may be one that was historically present at the site prior to the disturbance or based on nearby, undisturbed sites with similar ecological conditions. However, given the rapid pace of climate change and continued land-use change, it has been suggested to view the historical or contemporary reference ecosystem as a guide to work towards rather than a target (Higgs et al. 2014; Jones et al. 2018).

One of the first decisions when planning and implementing restoration activities is to identify where seed should be collected. The seed collection should aim to capture **genetic diversity.** Genetically diverse seed collections will maximise the capacity of the restored population to persist in the short term and respond to future environmental changes. Fundamental issues include whether seed should be sourced locally (termed **local provenance**) and whether such seed collections are genetically diverse or whether genetic diversity needs to be bolstered by mixing local seed with non-local seed collections. The choice to maintain a local only approach or mixing local with non-local seed will be context dependent (e.g. disturbance of the restoration site; see Box 1) and will likely vary between plant species and lifeforms (e.g. tree vs forb or grass).

In this module, we outline the various seed sourcing strategies to guide the selection of seed for restoration (Boxes 1 and 2). We begin by defining a seed **provenance** based on available information (geographic, genetic, and environmental), followed by an overview of current provenancing strategies, as well as their assumptions and risks. Then, we provide a decision tree to help identify which strategy might be best suited for the needs of the project. The choice of provenancing strategy will govern where to collect seed, and we introduce some of the desktop tools available to help guide these decisions. Finally, we provide guidance on the number of individual plants to sample within a provenance and conclude with consideration of some of the potential genetic and ecological risks associated with different provenancing strategies. This module assumes that the target species used in the restoration activity will be suitable for the site now and into the future. To this end, we provide only a general overview of how to predict the potential suitability of a species and options to investigate the suitability of similar species that are regionally native but not local to a site, but may offer the same ecosystem services and functions as the intended target species (Box 2).

Box 1. A landscape context to seed sourcing

The choice of seed sourcing strategy may depend on the landscape context – that is, where is the restoration site in relation to the seed source? If the restoration project is within a large area of undisturbed vegetation for which collection permits can be obtained, then, it is likely that it is appropriate to collect close to the site within that undisturbed vegetation (Panel A) (i.e. local provenancing – see section 'Which provenancing strategy should I use?'). For instance, a mine within an undisturbed area of forest or a degraded section of a large patch of remnant vegetation. However, a restoration project may have no nearby, large populations, but instead comprise several small populations within remnant vegetation (Panel B and C) (see section 'Strategies to mix local with non-local'). For instance, restoration of small remnants within a matrix of cleared agricultural or urban land. In this case, choosing where to source the seed is more challenging, and there will be a trade-off between maintaining locally adapted genetic variation that may be important in optimising establishment and growth, and maximising **genetic diversity** through mixing local and non-local populations. Caution should be taken when collecting seed from isolated remnants – the population could have gone through a **genetic bottleneck** and seed may be **inbred.** The seed may also be **hybridised** with other species, as has been found for isolated *Eucalyptus* trees (Field et al. 2011; Bradbury et al. 2016). However, the extent of inbreeding and reduced genetic diversity may be dependent on the mode of pollination (wind vs. bird vs. insect) and the **mating system** of the species (Krauss et al. 2007; Breed et al. 2015; van der Merwe et al. 2021).



Hypothetical example of a restoration site (hatched rectangle) and where one may collect seed for revegetation. (A) If a restoration site is within a large generally undisturbed area of native vegetation then seed can be collected locally. (B) If the restoration site is within a matrix of farms with remnant patches of native vegetation then seed can be collected at various distances away from the site to capture genetic diversity. (C) If the restoration site has only isolated paddock trees but there is a distant population (grey trees) of the same species then seed could be collected and mixed for the isolated trees and non-local population.

Box 2. Using species distribution models to inform species choice

If environmental conditions change, due to climate change, altered topography due to mining activities, or increased salinity due to land-use change for example, some species may no longer be suited to the local conditions. Common garden field trials (e.g. Gray et al. 2016; Bailey et al. 2021) can help determine if a species is suitable for a specific site or climate, however in the absence of such data, ecological niche models (ENMs; also referred to as species distribution models) may provide practitioners with a tool to estimate the extent of suitable climate habitat now and into the future (Butterfield et al. 2016; Harrison 2017). These models use the occurrence records of a species and relate their occurrence with climate, topography and soil and other environmental variables. This relationship is then visualised across the landscape as a spatial surface that represents the probability of habitat suitability. Several tools are available to assist with this approach (e.g. the Biodiversity and Climate Change Virtual Laboratory, http://bccvl.org.au/; Provenancing Using Climate Analogues, Harrison et al. 2017; Restore and Renew, https://www.restore-and-renew.org.au/).

While the outcomes of species distribution models should be treated with some caution, specialists are available to assist with the analysis and interpretation of results. It should also be noted that models make assumptions about the climate niche of a species, and do not consider that a species' distribution may be influenced by other important factors. If models predict the environment to be unsuitable in the future for a species, one option would be to substitute the unsuitable species with one which offers the same **ecosystem service and functions** or use a close relative of the target species (e.g. a subspecies or sister species). A comprehensive assessment should be undertaken when introducing a species into an area it does not occupy naturally to prevent unintended consequences (see Swarts et al. (2018)). **Demographic swamping** of nearby local species may occur if the introduced species has invasive potential. **Genetic swamping** could lead to local extinction when outcrossing species hybridise with a rare relative. Informed decision making regarding the introduction of non-local species into a restoration site should be made within a risk management framework (Byrne et al. 2011).

What is a provenance?

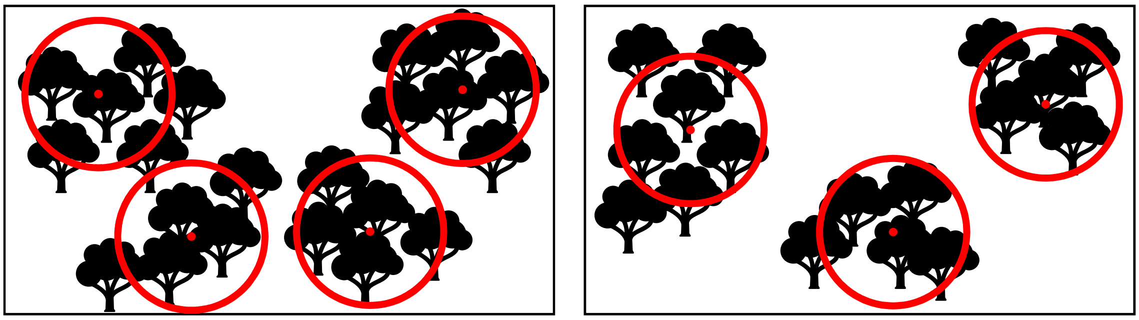
The term '**provenance**' refers to the geographic location of a seed collection, while a **population** refers to the group of individuals of the same species and their offspring (Rehfeldt et al. 1999). A **provenance** is scale and environment dependent, and genetic composition of the seed will be shaped by the life-history characteristics of the population (e.g. lifeform, longevity, reproductive and pollination biology, seed dispersal mechanisms) and the species evolutionary history (e.g. contraction and expansion of the distribution range during past climate change). For example, isolated populations of a naturally fragmented or confined species may each constitute a provenance, whereas for a species with a more or less continuous distribution, a provenance may be a number of overlapping populations (Figure 1).

How to define the extent of a provenance?

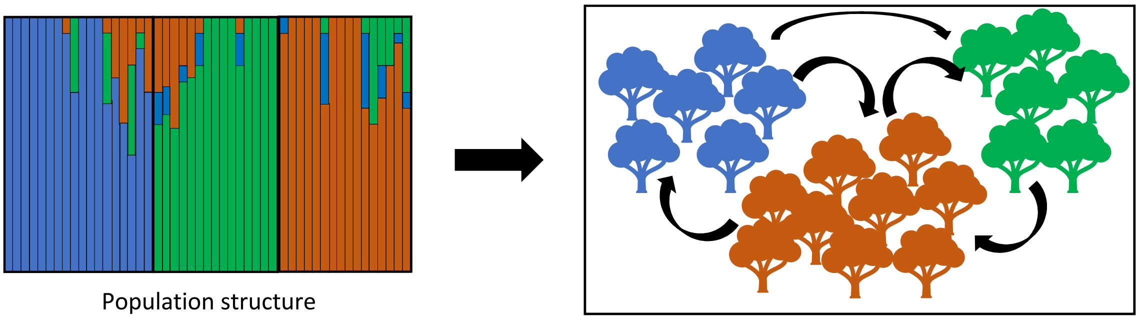
Geographic distance (geographic provenance)

There are many ways to define the extent of a provenance. The simplest approach is to use a geographical rule (Hancock and Hughes 2012) which we call a 'geographic provenance'. For example, in a hypothetical landscape, a geographic provenance may include all the individual plants of the same species within a 50 km radius (Figure 1A & B) which can be easily drawn on a map to indicate the provenance boundaries for that species. This approach seems logical, especially if the environment is very similar within the boundary. However, if there are large environmental changes within this boundary (e.g. changes in soil types or steep environmental gradients up a mountain side), this approach may not be suitable. When large environmental differences occur, plants may be adapted to their local conditions and may not survive if used for restoration when planted under different environmental conditions (Leimu and Fischer 2008). Additionally, the size and shape of the geographic provenance is likely to differ among species and, in part, depend on the mating system and the dispersal distances over which their pollen and seeds are dispersed (Martyn Yenson et al. 2021). Therefore, it is recommended not to generalise across related and co-distributed species based on an arbitrary radius (Rossetto et al. 2020).

(A) (Semi-)Continuous populations (B) Naturally fragmented populations



(C) 'Genetic population' defined by population structure



(D) 'Genetic population' defined by relationship among individuals

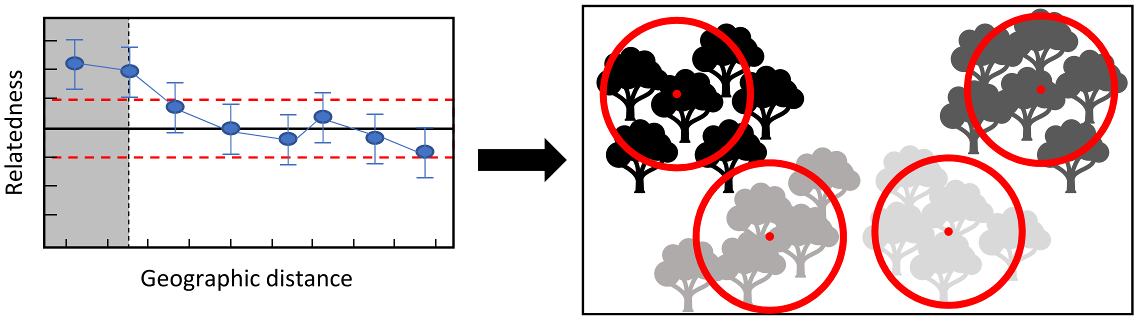


Figure 1. Examples of how a 'geographic' (A and B) and a 'genetic' (C and D) provenance could be defined. The red circles in A and B correspond to a hypothetical pre-defined geographic radius (e.g. 50 km) which delineates individuals into provenances for a species with a naturally (semi-)continuous (A) or fragmented (B) distribution. Genetic provenances can be defined through various approaches (molecular markers [C and D] and quantitative genetic traits [D]; but see text). Provenances can be delineated using population genetic structure that groups populations according to shared allele frequencies among individuals (C). Here, each colour in the bar chart (left hand panel of C) corresponds to a genetic provenance, and the shared affinities among populations (i.e. the mixed colours in a bar) represents past migration of seed/pollen (black arrows in right hand panel of C). A genetic provenance may also be delineated using statistical approaches, such as testing for the relatedness among individuals and relating this to geographic distance (D). In this example, the spatial correlogram (a plot of correlation statistics vs. geographic distance) shows individuals are significantly more genetically similar when they are geographically closer together (grey shading in left hand panel of D; based on the 95% confidence intervals around the correlation statistic falling outside the dashed red line) than individuals geographically further apart. This concept of spatial autocorrelation can be applied to molecular and quantitative genetic data to delineate the geographic extent of similarity (red circles in right hand panel of D).

Environmentally-informed geographic distance (environmental provenance)

Environmental criteria can be imposed to define locally adapted seed sources, hereafter defined an 'environmental provenance'. Here, habitat matching of the same species at the seed source and restoration site provides insight into provenances inhabiting similar environmental conditions. Presumably, these habitat-matched sites support similarly adapted plant populations. Practitioner knowledge of soil and climate variation across a species distribution can provide an intuitive sense of matched habitats. However, powerful web-based tools and datasets are also available to provide a more sophisticated approach. For example, Harrison et al. (2017) and Doherty et al. (2017) present methods that use climate data to calculate a climate similarity index between two sites. This index can be used to predict relative performance of seed at a target site under current climate conditions. From this, a species-specific seed transfer map based on climate (as well as provenance fragmentation, in the case of Harrison et al. 2017) can be generated for available seed sources.

Genetically-informed geographic distance (genetic provenance)

A provenance can also be defined by associating genetic similarities among individuals with geographic distance, here termed a 'genetic provenance'. The pattern of genetic variation in plants and how this variation is influenced by **gene flow** is a function of pollen and seed dispersal. These mechanisms, in combination with the **mating system**, have a major influence on the distribution of genetic variation or **population structure** (Figure 1C) among plant populations (Hamrick et al. 1996). High levels of **gene flow** generally occur in obligatory or highly outcrossing species, and those with mechanisms for long distance pollen and seed dispersal such as small pollen or winged seed. The size of the genetic provenance will in part reflect the size of the family groups (e.g. group of related individuals) and **gene flow** (e.g. dispersal of pollen or seed). Some examples of the size of a genetic provenance based on genetic studies are provided in Table 1. There are several initiatives underway that aim to make this information available for practical use. For example, Restore and Renew (Box 3; Rossetto et al. 2019; Rossetto et al. 2020) provides provenance boundaries for restoration of specific sites based on genetic diversity estimates of **gene flow** across the landscape, combined with environmental data. In this case, the genetic provenance boundary is site and species specific, and based in empirical data.

Genetic information can be obtained through various ways. The two most common approaches are to use morphological traits (i.e. **quantitative genetic traits**) or **molecular markers** (i.e. population genetics). The first approach looks for population differences in morphological and functional traits, for example differences in relative leaf shape and size, and associated changes in the traits among populations with geographic distance (Figure 1D). Such an approach was applied by Harrison (2017) who used population variation in early-age functional traits to detect a general geographic extent of 27 km for *Eucalyptus ovata* in Tasmania, Australia (Table 1). The second approach uses molecular markers (i.e. a variable fragment of DNA), such as **microsatellites** or **single nucleotide polymorphisms** (SNPs). While the technical detail behind molecular markers is not important for these guidelines, it is worth noting that different molecular markers will provide insight on different evolutionary processes. For example, microsatellites are sections of a species genome which are generally not under **natural selection** and thus provide information on barriers to **gene flow** or dispersal (Mijangos et al. 2015). Multiple microsatellites were used in a study of *Eucalyptus pauciflora* to associate the relatedness among individuals with geographic distance and define the geographic extent of a local provenance (Figure 1D; Table 1; Gaul et al. 2014). Other genome-wide markers, such as SNPs can provide information on genomic signals of adaptation to the home-site environment (Breed et al. 2019).

Table 1. Examples of studies that have employed quantitative genetic traits or molecular markers to delineate the geographic extent of a provenance using breaks in relatedness among individuals. The geographic distance of a genetic provenances varies across genera and even within genera (e.g. *Eucalyptus* and *Grevillea*), and may reflect the evolutionary history of the population or how pollen and seed is dispersed (e.g. insect vs. bird, wind vs. gravity). In the Australian flora, range disjunction and abundance also have significant effects on genetic differentiation (Broadhurst et al. 2017). However, the differences could also in part be associated with the different molecular markers used. Nevertheless, this variation in geographic distance highlights the importance of considering genetic information when defining a provenance.

Species	Genetic approach	Geographic distance of a provenance (km)	Citation
Eucalyptus ovata	Quantitative genetics	27	Harrison 2017
Eucalyptus pauciflora	Population genetics	27	Gauli et al. 2014
Eucalyptus melliodora	Population genomics	500	Supple et al. 2018
Banksia menziesii	Population genetics	30	Krauss et al. 2013
Dryandra lindleyana	Population genetics	20	Krauss and Koch 2004
Mirbelia sp. Bursarioides	Population genetics	59	Millar et al. 2015
Melaleuca nematophylla	Population genetics	212	Millar et al. 2015
Grevillea paradoxa	Population genetics	386	Millar et al. 2015
Grevillea globosa	Population genetics	27	Millar et al. 2015

Box 3. Restore and Renew

A decision support system for knowledge-based ecological restoration, vegetation management and species conservation.

Restore and Renew Webtool https://www.restore-and-renew.org.au

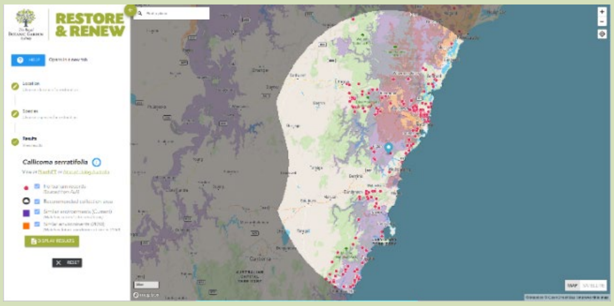
The freely available information on the Restore and Renew webtool can be used to design a seed collecting strategy. The information provided is easy to access and easy to interpret, and is based on the location of a user-selected restoration site. For species with available genomic data the collection strategy can be based on case-specific **genetic provenance boundaries** and **climate boundaries** that can be adopted within any restoration scenario.

Box 3. Restore and Renew continued

Using the webtool for: Site and species specific genetic provenance boundaries

1. Go to https://www.restore-and-renew.org.au to enter the webtool,
2. Enter location **details** and **select a species** (currently mostly NSW) from the drop down menu,
3. Select type of results (default without future climate data),
4. Wait while calculations for climate envelopes and genetic provenance are made on the fly (based on background information provided by the Restore and Renew knowledge infrastructure),
5. Inspect the results on the screen, zoom in and out to obtain precise locations and download the results as a PDF that also contains additional information on the species and on how to optimise genetic diversity at a site for a range of strategies.

The genetic provenance is calculated based on measured natural levels of gene flow and connectivity which are then used to define the area within which material (e.g. seeds) can be sourced to maximise diversity without bringing together plants that are too genetically dissimilar. The prediction is based on genomic data from species-wide collections using the DNA from many individuals across many sites.



Example: *Callicoma serratifolia*
Blue teardrop = selected site; non-shaded area = site specific genetic provenance; Purple areas = current climate match; orange areas = future climate match; red dots = herbarium records.

Using the webtool for: Site specific (current and future) climate matching tool

Within this additional webtool, selecting a site allows users to see the distribution of environments closely matching the selected site under current climate conditions. This allows users to consider collections which may accommodate effects of local adaptation. Users can also display the distribution of environments which are present in the landscape now but which are predicted to occur at the selected site under climate change. This can inform the implementation of restoration strategies incorporating "future proofing" approaches.

Customised or specific projects:

Using methodology developed for Restore and Renew the data are also used for targeted projects such as:

- Developing seed production areas (maximising diversity and minimising relatedness),
- Supplementing current seed collections to increase diversity and/or for targeted representativeness,
- Introducing specific genetic traits (e.g. resistance, climate proofing) within a balanced genetic background,
- The development of a specific toolkit for threatened species (e.g. translocations based on optimising genetic diversity).

Which provenancing strategy should I use?

Local or mixed provenancing?

Irrespective of how the extent of a provenance is defined (geographic, environmental, or genetic; see above), restoration programs have traditionally followed a local provenancing strategy using seed from plants collected on-site or close to the restoration site. While local provenancing is widely used (e.g. Hancock and Hughes 2012; but see Hancock et al. 2020), the definition of 'local' varies. Some define it as within the watershed which can extend to hundreds of kilometres, for others it's defined as a geographic distance (radius of 5-20 km; see "Geographic distance" above) and may or may not be species specific (Hancock and Hughes, 2012). Nevertheless, the use of local seed has some advantages. First, it is assumed that seed from these provenances will be best suited to the local conditions and have a '**home-site advantage**' due to being **locally adapted** to climatic, soil, pest and pathogens and other environmental conditions (Grady et al. 2015). Secondly, a local provenancing strategy limits the potential of **outbreeding depression**, which is the reduction in fitness of progeny relative to the parents following the intra-specific breeding between local and non-local plants (Costa e Silva et al. 2012; Hufford et al. 2012). It will also reduce the likelihood of **heterosis**, in which the hybrid progeny formed by breeding between the local and non-local plants show greater vigour than the parents (Costa e Silva et al. 2014), which could lead to **demographic swamping**. Thirdly, it assists in maintaining the co-evolutionary history of the plants within the region (Grady et al. 2017; Camarretta et al. 2020) and the broader community of the ecosystem (Whitham et al. 2006; Gosney 2017). Lastly, it limits the risk of bringing in non-local seed that may not be adapted to the local environment (site **maladaptation**), which decreases the chances of establishment success (Grady et al. 2015).

The local provenancing strategy has been challenged as it may no longer be optimal in a rapidly changing world (Broadhurst et al. 2008; Jones 2013; Breed et al. 2018). Land-use change has increased disturbance and fragmentation of remnant vegetation. This change can decrease genetic diversity within a population (Young et al. 1996) and increase selfing (inbreeding) rates (e.g. Gauli et al. 2013). Inbreeding can result in the reduced fitness of offspring due to **inbreeding depression**, especially for naturally outcrossing plants, and its effects are often accentuated when inbred offspring are exposed to stressful growing conditions (Nickolas et al. 2019). Furthermore, climate change is expected to change the conditions that have historically shaped local adaptations at the restoration site (e.g. Etterson 2004; Costa e Silva et al. 2018; Costa e Silva et al. 2019). While in some cases local populations may persist through changes in **phenotypic traits** without changing their genome (**plasticity**), there will be a limit to such phenotypic changes. Local genotypes therefore may be maladapted to the new climatic conditions. More genetically diverse seed collections may provide the higher levels of genetic variation and therefore capacity for adaptation, population survival and long-term population persistence in an uncertain future.

To this end, several provenancing strategies have been proposed. These strategies, with the exception of one, supplement local seed collections with seed collected from non-local provenances with the general aim to increase genetic diversity and the potential capacity of the population to evolve to new environments (**adaptive potential**). This could improve the resilience of restored populations, species and ecosystems under contemporary and future environmental change.

Strategies to mix local with non-local

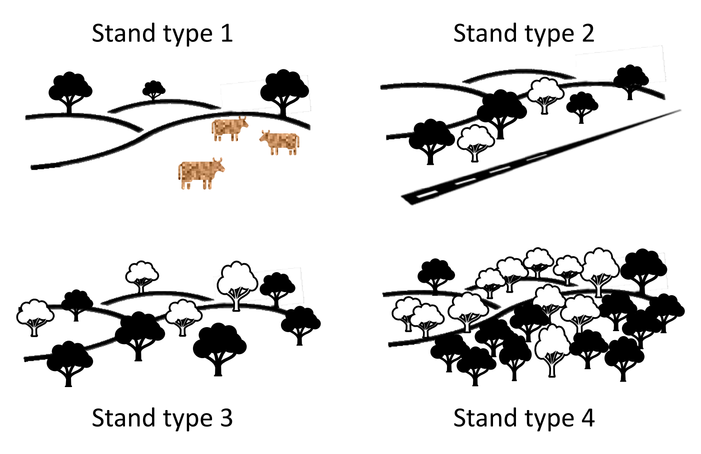
There are several provenancing strategies aimed at mixing local with non-local provenances. To help decide which provenancing strategy might be best suited to the restoration project, we provide a generalised decision tree that steps through some of the possible questions to guide the decision process on choosing an appropriate alternative provenancing strategy (Figure 2). This decision tree considers the state of the local provenance (i.e. whether seed can be sourced locally, current genetic diversity) and the site being restored (i.e. degree of disturbance, fragmentation, and climate change). This is by no means an exhaustive list of questions one might ask as some situations will be context dependent and the integration of more than one strategy might be best applied under a given circumstance. It is important to note that one provenancing strategy may not apply to all species or lifeforms, for example, in case where species have short generation times. We explain how to implement each strategy, and detail some of the tools that are available to help identify candidate non-local provenances. Some strategies require prior knowledge of the species to implement (e.g. estimates of **gene flow**) and we attempt to detail the various sources of data that may be needed or used as proxies from closely related species.

Irrespective of which provenancing strategy is employed, it is essential that excellent records are kept for each provenance (e.g. the location of the collection, the number of individuals sampled and the size of the collection area, the health, size, and level of fragmentation of the provenance etc.; see Module 4 – Record keeping) and that each provenance is maintained as a separate seed collection (Box 4). Further, if feasible, it is recommended to use these strategies in an experimental framework, through the embedding of experiments among the broader plantings to provide an opportunity to learn and refine these strategies (Breed et al. 2013; Broadhurst et al. 2017; Gellie et al. 2018; Bailey et al. 2021). Indeed, such an approach is beginning to reveal thresholds to upslope migration (Camarretta et al. 2020) and performance differences among provenances signalling the extent of local adaptations (Breed et al. 2016; Costa e Silva et al. 2019), in the case of some *Eucalyptus* species.

Box 4. Opportunistic seed collections

Seed may be collected without a specific restoration target or known client. In such cases, seeds are collected opportunistically, and there are several important points to note. Firstly, make sure to collect from many plants (See Table 2), as collections from one or two plants are generally of limited value in terms of evolutionary capability and potential. Secondly, always record the location of the collection, the number of individuals sampled and the size of the collection area, the health, size, and level of fragmentation of the provenance (see Module 4 – Record keeping). Collect seed from individuals that are wide-spread and healthy, and try to collect equal numbers of seed from separate plants. Most importantly, store seed from different provenances separately.

When buying seed, how should seed lots be selected if there are a limited number of seed lots available, none of which are close to the planting site? In such a case, try to select a seed lot from an area with the most similar environmental parameters such as rainfall, temperature, and soil type. Otherwise, follow the decision tree in Figure 2 to help select a non-local provenance. Irrespective, answers to this question depends on having good records attached to each seed lot. If the precise collection location of the seed lot is available, the climate averages, soil types and topography can be determined from a map or other spatial data.



Stand type classifications reflecting different levels of fragmentation across a landscape. Stand types correspond to: isolated paddock trees (1); a small patch of remnant vegetation (2); open stand of continuous vegetation such as a eucalypt woodland (3); and a closed stand of continuous vegetation (4). These classifications have been used to study the effect of fragmentation of genetic diversity (e.g. Gauli et al. 2013). (Figure modified from Borralho and Potts 1996)

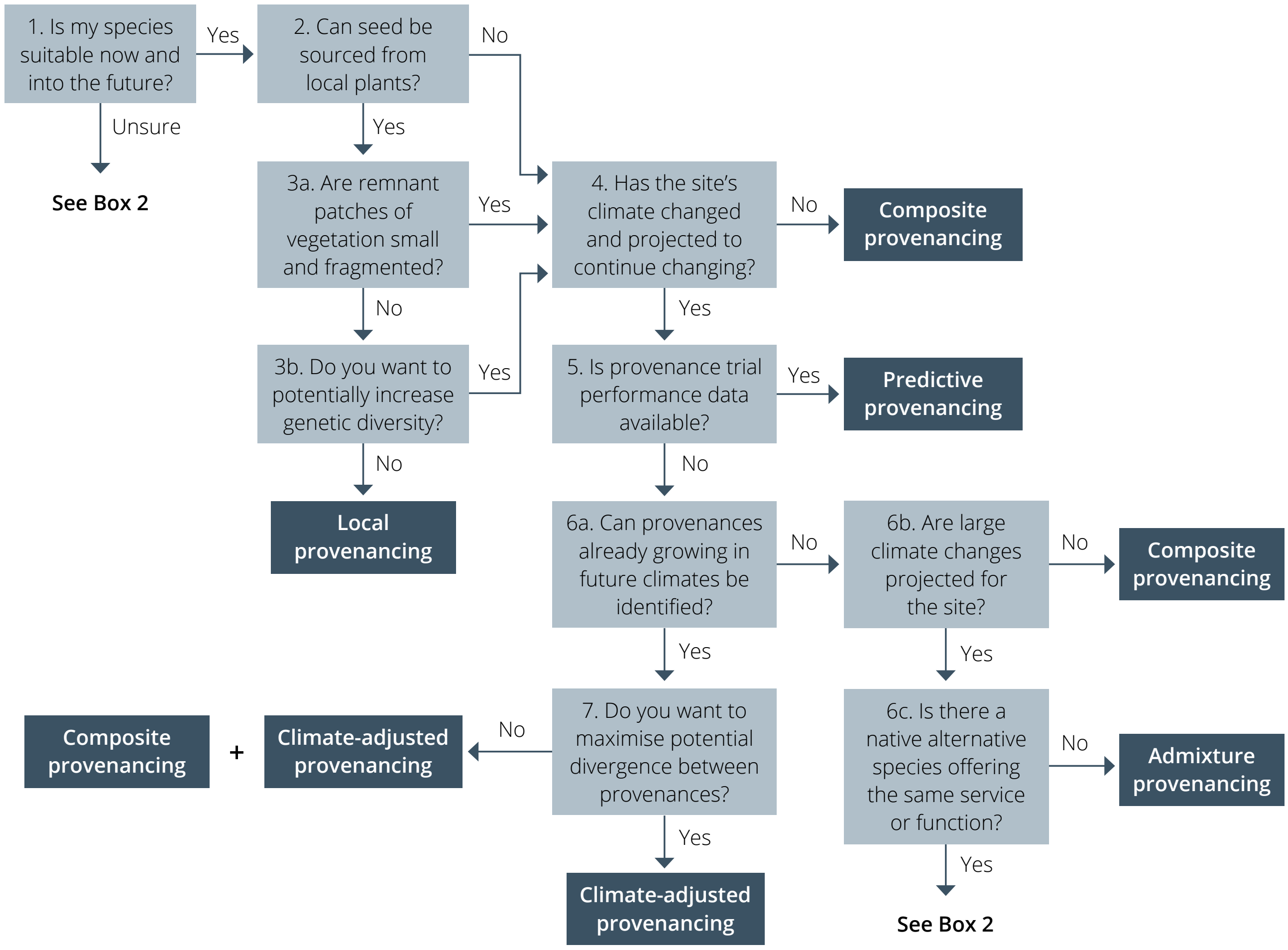


Figure 2. A generalised decision tree for choosing a potential provenancing strategy given the state of the local provenance and the site being restored. The decision tree gives a non-exhaustive list of questions one may ask when determining which provenancing strategy best matches requirements.

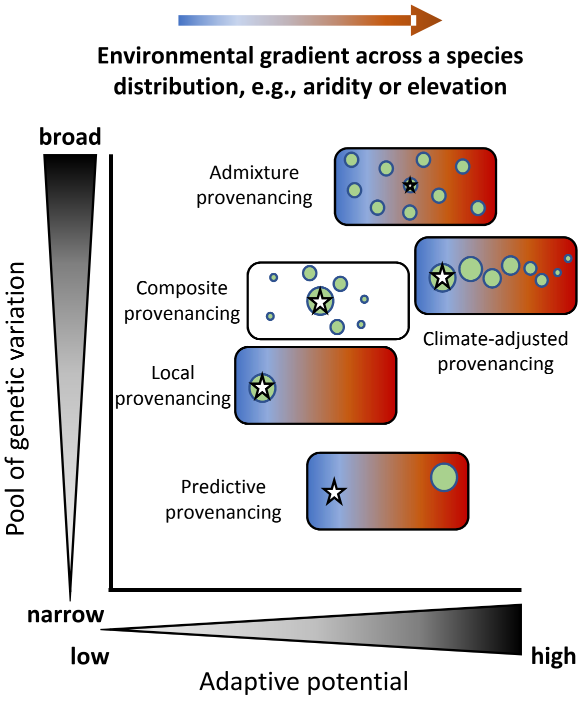


Figure 3. The potential pool of genetic variation and adaptive potential of local and mixed provenancing strategies. Each strategy is depicted within a hypothetical landscape, where the colour gradient corresponds to an environmental gradient across a species distribution, such as elevation or aridity. The site being restored at the margin of this hypothetical landscape is identified by the white star and the green circles correspond to native provenances across a species distribution range. The size of the circle indicates the relative sampling effort within a provenance. Note that the application of composite provenancing and admixture provenancing requires no knowledge of any environmental change and that the selection of provenances under these strategies would extend on either side of the white star. The position of each strategy corresponds to the potential trade-off between the genetic gain (i.e. pool of genetic variation) and adaptive potential (e.g. the population's ability to respond to environmental changes) of the established planting. (Figure modified from Prober et al. 2015)

Composite provenancing

Composite provenancing mixes seed from local and non-local provenances to increase local genetic diversity (Figure 3) but does so in a way to mimic natural **gene flow** among populations, noting that this strategy is not restricted to collecting along a climate gradient. This strategy mixes local seed with seed collected from non-local provenances that are geographically close to the restoration site, and a reducing amount of seed from provenances further away from the site (Figure 3). A seed mix, for example, could comprise a mixture of 50% local seed, 40% from adjoining non-local provenances, and 10% from provenances further away. The effective deployment of this strategy relies on information about the extent of **gene flow** among provenances. However, the extent of **gene flow** is poorly understood for many species, including those used in ecological restoration. While information on **gene flow** can be determined from genetic studies using molecular markers (e.g. using microsatellites or SNPs; see Box 3 for selected east coast species) to define the extent of a genetic provenance (see above and Figure 1), undertaking such studies may be outside the scope of the project. In such cases, the extent of **gene flow** could be estimated using a geographic or environmental provenance definition or using gene flow estimates from related species with a similar population structure as a proxy.

Admixture provenancing

Admixture provenancing seeks to increase genetic diversity by sampling from multiple provenances across the species distribution (Figure 3 - noting that this strategy is not restricted to collecting along a climate gradient; Breed et al. 2013). It can be applied as a 'bet hedging' strategy if the direction and/or magnitude of future climate change are unknown, as genetic diversity is pooled from across the entire range of a species (Breed et al. 2013). This strategy supplements equal quantities of local seed with seed from non-local provenances across the species native range (Figure 3). However, there are some risks as the non-local provenances need to establish under the contemporary conditions experienced at the site being restored, thus there is a risk of early-age maladaptation (Grady et al. 2015). Additionally, if provenances are mixed, but have been historical isolated from one another (e.g. through a range disjunction), there is the risk of **outbreeding depression**.

Predictive provenancing

The predictive provenancing strategy seeks to match the seed source with a current site that has a predicted future climate scenario for the restoration site (Figure 3; Sgro et al. 2011). If performance data (e.g. later-age growth, reproduction, and survival) of multiple provenances grown in a common garden trial(s) are available, this can inform a predictive provenancing strategy to predict which single provenance may be best suited to the current and future climate of a restoration site based on its performance when grown under such conditions (Crowe and Parker 2008). This strategy potentially offers an alternative that maximises the **adaptive potential** of the plants at that site, but at the cost of having a narrower pool of genetic variation, which could lead to **maladaptation**.

There are several drawbacks of this strategy. Firstly, this strategy prescribes the use of seed from the identified provenance(s) without the option to mix the non-local seed with locally collected seed, thus the **evolutionary history** of the local provenance is potentially lost or diluted. Secondly there is a risk that the non-local provenance is not adapted to the current conditions, i.e. it may be predicted to be optimal under future climates but the provenance needs to establish under current climates (Grady et al. 2015). Thirdly, if predicted environmental changes that underpin the selection of the non-local provenance turn out to be incorrect, then the non-local provenance may perform worse than if the site was planted with only the local provenance. Lastly, the long-term field trial experiments providing performance data that can be used to implement this strategy are rarely available, and indeed are generally only available for commercially important forestry species (e.g. Dutkowski and Potts 2012; Gray et al. 2016).

Climate-adjusted provenancing

Climate-adjusted provenancing is similar to predictive provenancing in that it seeks to account for changing climate, but contrasts because this strategy targets provenances along a gradient of future change (e.g. gradient of low to high aridity). The premise of this strategy relies on two assumptions. First, it assumes that genetic variation among provenances has been shaped through natural selection by the home-site climate. This assumption is generally well supported by many studies that have looked for signals of climate adaptation across climate gradients (morphology: Gauli et al. 2015; Harrison 2017; genomics: Steane et al. 2014, 2015, 2017; Ahrens et al. 2019). Second, as with predictive provenancing, it assumes that non-local provenances matched to future climates of a restoration site can successfully establish under current climate conditions. If these assumptions are met, this strategy may provide greater **adaptive potential** and a greater pool of genetic variation for future selection events (e.g. drought or heat stress).

Identifying provenances for this strategy is relatively straightforward. If the direction of climate change is known, then selection of provenances is simply along that climate gradient. For example, if the restoration site is projected to become warmer than present by the end of the century, seed is collected from provenances that currently occupy environments that are increasingly warmer than the contemporary climate at the site, with the amount of seed used from non-local provenances reducing along the gradient (Figure 3). This provides a seed mix with a range of adaptive variation to cater for the uncertainty in the magnitude of change in future climates. If the direction of climate change is unknown, it can be estimated through the analysis of species occurrence data and future climate projections.

In most cases, species occurrence data is available through the Atlas of Living Australia (ALA), but keeping in mind that some occurrence records might be miss-identifications or obvious ornamental plantings outside the known distribution range of the species. Similarly, contemporary and future climate data can be freely downloaded from various databases, including TERN, WorldClim, and CHELSA. Once the required data is available, there are numerous desktop applications available to help guide the choice of provenances. Methods for detailed analysis and identification of provenances are provided in Harrison et al. 2017, Ramalho et al. 2017; Hancock et al. 2018; Rossetto et al. 2019.

Once climate-adjusted provenances are identified, there are various ways in which to mix the non-local seed with the local seed. Harrison et al. (2017) provides some discussion on the potential ways to mix seed following this approach. One of these approaches is to use the uncertainty in the future climates as a guide. For example, there is generally good agreement among models of the future climate up until the mid-century (e.g. 2050s). After this period, the predictions by the models vary substantially (especially for precipitation). Taking this variation into account, one possible seed mix may comprise 50% of local seed, 25% from provenance matched to the early-future climate, 15% matched to the mid-century climate, and 10% matched to the end of this century climate. This mix can be further tailored to minimise potential differences among provenances by mixing provenancing strategies, such as climate-adjusted provenancing with composite provenancing (Figure 3).

Practical considerations

If the restoration site involves tens to hundreds of species, it may be challenging to implement a specific provenance strategy for each species. Also, seed availability may restrict the options available. Having clear goals and objectives (Module 1 – Introduction), allowing plenty of time for the planning phase, possibly multiple years for seed collection, and prioritising species which will be subject to an alternative provenance strategy may assist.

In many cases, however, you will have to make pragmatic decisions to collect the best quality seed you can. Where resources are limiting and only small amounts of seed are required, it is best to focus on maximising genetic diversity. The best way to do this is by ensuring that seed is collected from as many plants as possible that are well-spaced to minimise sampling related individuals. As tempting as it is to collect from one or two loaded, low-hanging branches, it makes future plantings from this seed vulnerable to failure as the seed may be inbred or have low genetic diversity.

How much to collect?

How much seed can be collected from within a population will likely be governed by the collecting permit (see Module 3 – Approvals, Principles and Standards for Seed Collection). Each state or territory has different permit conditions regarding the amount of **germplasm** that can be collected at any one time. Allowing the collecting of no more than 20% of reproductive material from a single plant is common. Consideration also needs to be given on the number of times individuals within a population are re-collected within a given period to limit collection attrition (i.e. over collection of seed) and depletion of the seed bank, which is critical for the regeneration of the next generation in the case of a disturbance (e.g. fire) (see Module 6 – Seed Collection). This is particularly important in cases where multiple collectors harvest reproductive material from the same provenance, such as where a particular site is readily accessible.

There is little doubt that the quantity of seed required for restoration programs is often enormous, and ongoing disturbance such as land-use and climate change will likely add pressure on plant reproductive capacities, limiting the availability of seed (Broadhurst et al. 2015; Broadhurst et al. 2016; Merritt and Dixon 2011). In such cases and to limit the reduction in seed availability through over collecting, the establishment of seed production areas (see Module 7 – Seed Production) for key species widely used in restoration programs or for provenances identified by climate-adjusted provenancing across multiple jurisdictions may help generate and safeguard seed supplies for future projects.

In this section, we give a brief consideration to the number of provenances and individuals within a provenance from which to collect seed, reflecting on the various provenancing strategies mentioned above. The extent of the seed collection will no doubt be determined by budget constraints and the provenancing strategy employed, among other factors. However, we recommend to broadly represent the standing genetic diversity within provenances where possible.

Number of provenances for each strategy

Often, there is a trade-off between the number of provenances and the number of individuals sampled within a provenance. For example, if composite provenancing is planned for in a restoration program, then the emphasis may be on collecting from more individuals within a provenance but fewer provenances. However, if admixture provenancing is considered then we may want to sample fewer individuals within a provenance but more provenances. The number of provenances to sample will be dependent on the provenancing strategy and number of provenances that exist for a species. It is important to note that the number of provenances may also depend on the lifeform (e.g. grass vs. tree) and the species itself (e.g. rare vs. widely distributed). Additional considerations for seed sampling may apply to rare and threatened species (Germplasm Guidelines Chapter3; Translocation Guidelines). If you are purchasing seed or plants from a supplier, we recommend discussing with the supplier your selected provenancing strategy so that together you can identify the best provenances and how much to purchase for each provenance (e.g. see the end of each provenancing strategy ‘Strategies to mix local with non-local’ for an example on provenance mixing).

Keeping in mind the above caveats and the objectives of the selected provenancing strategy, sampling of a provenance should always be undertaken to maximise the genetic diversity whilst meeting the objectives of the restoration project. This can be achieved by collecting seed evenly from many individuals across the whole population. Below we provide a suggested minimum number of provenances which could be included following each provenancing strategy (Table 2), keeping in mind the limitations of seed availability, number of provenances for the target species, and budget.

In the case of composite provenancing, it is suggested that seed from at least three non-local provenances be mixed with seed from the local provenance, resulting in a seed mix comprising four provenances. In this case, the choice of non-local provenances will be dependent on a number of factors, including disturbance within the selected non-local provenance and proximity of the non-local provenance to the local provenance (or restoration site). One suggestion may be to collect seed from two provenances which adjoin the local provenance and one provenance that is further away but still within the estimated **gene flow** distance (if known).

For climate-adjusted provenancing, it is suggested that at least five non-local provenances currently occupying environments along a gradient of future climate projections for a given site being restored be mixed with the local provenance. In the case of admixture provenancing, the sampling of provenances will be influenced by how provenances are distributed across the landscape and whether the species has a restricted or widespread distribution. If provenances are naturally small and disjunct, then a seed mix may consist of most of the known provenances of the species. If the species occurs across a homogenous environment, then a smaller sample of provenances may be adequate. However, if the species occupies a landscape with steep environmental gradients (e.g. a mountain side or a valley), it may be important to sample broadly to maximise **adaptive potential**. Nevertheless, a sampling design for admixture provenancing may consider 10-15 non-local provenances well distributed across the species distribution in as many different environments as possible.

Table 2. Suggested number of individuals to sample within each provenance for three alternative provenancing strategies. The number of provenances to sample for each provenancing strategy corresponds to the suggested minimum sampling strategy from the 'Number of provenances for each strategy' section. The number of individual mothers to sample within each provenance represents the upper whisker of the box plots in Figure 1 of Hoban (2019). The amount of seed per sampled individual should not exceed 20% of the crop.

Provenancing strategy	Suggested number of provenances (including local)	Minimum number of individuals from each provenance to capture locally common alleles	Minimum number of individuals from each provenance to capture 95% of all possible alleles
Local	1	16	125
Composite	4	14	100
Climate-adjusted	6	13	80
Admixture	11-16	12-10	50-40

Number of individuals from which seed should be sourced

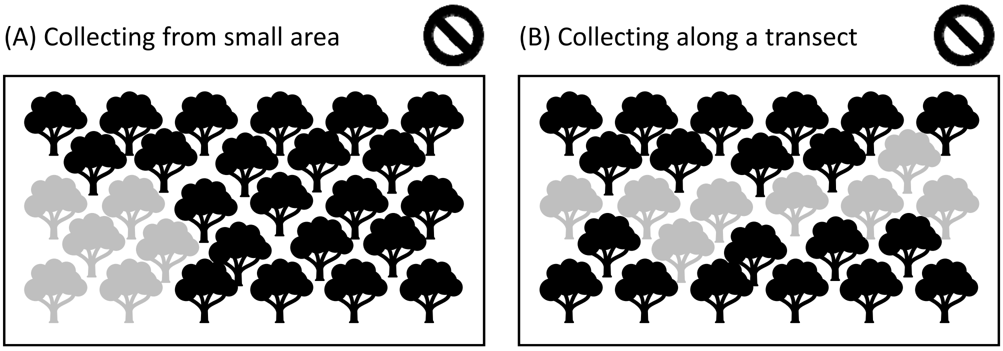
The number of individuals to collect seed from within a provenance is an important consideration, as collecting too few individuals may result in unintended and potentially negative genetic effects on the next generation via a genetic bottleneck (i.e. a reduced representation of the genetic diversity). For example, a recent review found that for more than half of the studies reported, genetic diversity was reduced in the planted progeny relative to the original provenance from which the seed was sourced (Jordan et al. 2019). Although there exists a trade-off between collecting intensity and cost, the main goal is to represent the genetic diversity within a provenance as best as possible. Genetic diversity of a seed collection increases with every individual plant added to the collection. It is therefore better to collect small amounts of seed from many well-spaced individuals rather than large amounts of seed from few individuals or close-by individuals. Brown and Marshall (1995) advised a general 'rule-of-thumb' to sample at least 50 or more individuals within a provenance to capture at least one copy of each **allele** (i.e. the possible forms of a gene) with a minimum number of 10-20 seed per plant and a maximum amount of 20% of the available seed per plant. While this rule of thumb is widely used

(Guerrant et al. 2004) and has value in its simplicity, Hoban and Strand (2015) have argued it is based on unrealistic assumptions about the provenance (e.g. each provenance being genetically distinct from one another) and recommend caution when applying such rules.

A recent study that developed a computer model which simulated the **genetic diversity** of multiple populations for a hypothetical species revealed a general pattern; the number of individuals to sample within a provenance decreases as the number of sampled provenances increases (Hoban 2019). In the absence of any knowledge on **gene flow** or effective population size, the simulation study by Hoban (2019) can be generalised to provide some suggestions on the number of individuals to sample within a provenance given a provenancing strategy (Table 2). If the goal is to capture potential local adaptations within a provenance, then between 10-16 individuals per provenances may be enough to achieve this goal. However, if the goal is to capture all possible copies of each **allele** and thus potentially maximise the genetic diversity of the restored population to future disturbances, then between 40-125 individuals per provenance may be more appropriate (Table 2). It must be noted, these are suggested number of individuals and the realised number of individuals to sample from within a provenance will vary species to species, depend on budget and time constraints and, to an extent, depend on the lifeform and mating system. Indeed, if the species has a **self-compatible mating system** (i.e. pollen from the same individual can fertilise ovules from the same individual or related individuals can mate and produce viable offspring), then it may be advisable to double the number of individuals sampled within a provenance but make sure that individuals are well spaced to avoid collecting from one family (see van der Merwe et al. 2021). It is also important to note that these suggested minimums (Table 2) do not consider a range of factors such as post-collection effects on *ex situ* seed collections where seeds may lose viability during storage and use of a collection (Module 9 – Seed Drying and Storage; Guerrant et al. 2004; Hoban and Strand 2015, Cochrane et al. 2009).

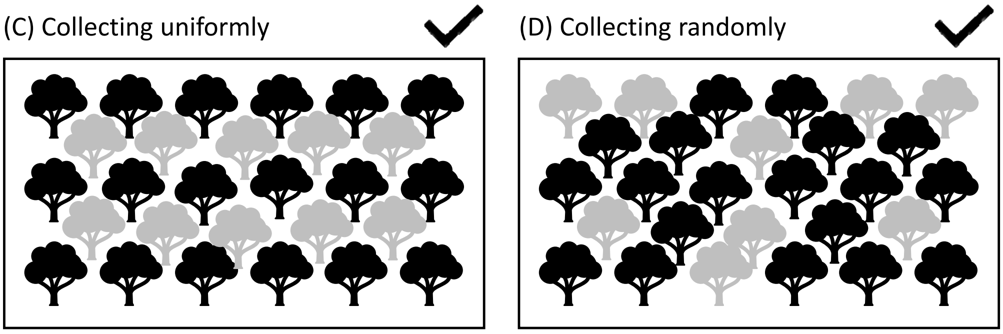
Sampling distances between individuals

The individual sampling strategy within a provenance influences how much genetic diversity is captured. That is, if seed is collected from one small area, rather than representatively of individuals across the whole population, then less genetic diversity will be captured. Simulation studies have shown that collecting within a small area of a provenance or along a transect resulted in less diversity compared to a random collection across the whole extent of the provenance or sampling across a uniform grid (Hoban and Strand, 2015; Figure 4). This may be because individual plants that are next to each other are more likely to be related (full siblings or half-siblings) compared with individuals that are further apart, as pollen is more likely to be transferred to immediate neighbours, and seeds are more likely to be dispersed nearer to the mother plant. Equal amounts of seed should be collected from individuals, avoiding over collecting from individuals that produce large amounts of seed. Collections from plants that show clonal reproduction (through apomixis or vegetative reproduction) should cover a large area and restrict seed collection to fewer individuals per site.



(A) Collecting from small area

(B) Collecting along a transect

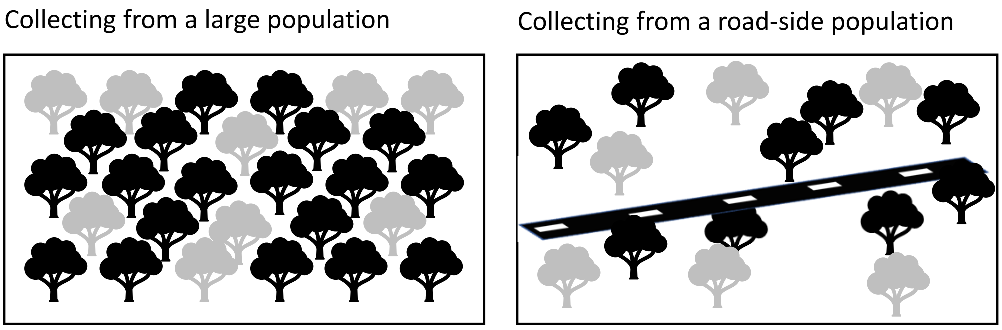


(C) Collecting uniformly

(D) Collecting randomly

Figure 4. Common collection strategies within a population. To maximise genetic diversity, it is recommended not to collect from a small area (A) of the population or along a transect (B), but rather to collect seed from individuals in a uniform pattern (C) e.g. equal space between individuals) or randomly (D) across the population. This limits the chance of collected seed from individuals that are related to one another and thus increasing the pool of genetic diversity. Collecting across the population may mean a little extra walking or driving, but the extra effort should result in a better-quality seed collection. Grey trees correspond to individuals where seed has been collected.

How individuals are collected within disturbed sites, such as a roadside verge or a population fragmented across a farm matrix, will differ from how you would sample a continuous or intact population (Figure 5). For example, if collecting seed from a roadside verge population that stretches for 4 km, instead of collecting from every individual along a 1 km stretch of road, perhaps collect seed from individuals randomly across the population keeping in mind distances between individuals.



Collecting from a large population

Collecting from a road-side population

Figure 5. Collection strategies within a population might differ depending on the level of disturbance, for example, collecting from a large population or a fragmented roadside verge population. In both cases, sampling randomly (e.g. the grey trees) while keeping appropriate distances between individuals will maximise the genetic diversity of the collected seed.

Final note

Decisions surrounding seed sourcing can be very complex, particularly when also accounting for constraints such as seed availability, time, and funding. One of the most important take home messages from this module is that there are clear benefits for the short- and long-term success of restoration by increasing genetic diversity in the seed collections of provenances according to the adopted provenancing strategy. It is recommended that seed collections from different provenances are kept separate, and that sufficient information on each collection is well documented, such as location information and site environmental conditions. Combined, these simple but highly important steps will enable flexibility when applying different provenancing strategies, in addition to enabling experimental assessment of provenancing strategies embedded in restoration activities. Finally, seed sourcing strategies need to be well documented (e.g. reason for a particular strategy, how provenances were chosen, mixing ratios of local and non-local seed, etc.) and shared among all stakeholders involved in the restoration project. Updating and reporting the outcomes of different provenancing strategies will ensure knowledge can be passed on to others in the sector and strategies can be refined to maximise restoration outcomes.

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Glossary

Adaptive potential: The potential ability of the population to adapt to new environments.

Allele: The possible variants of a gene or other region of DNA. The number of unique **alleles** is one measure of genetic diversity (see Genetic diversity).

Clonal reproduction: The production of genetically identical individuals by vegetative propagation or by apomixis (producing seeds that are genetically identical to the maternal plant).

Delineate: To mark or indicate the border or boundary.

Demographic swamping: Where one provenance (e.g. non-local) reproduces viable offspring quicker which excludes the establishment of offspring from another provenance (e.g. local).

Ecosystem service or functions: The benefits to humans provided by ecosystems. Examples are clean soil, water, air, and food.

Effective population size: The number of individuals in a population that contribute offspring to the next generation. This can be determined by counting all individuals within a population but in a population genetics context, it is the probability that two copies of a gene will be passed onto the next generation. The population genetic definition of an effective population size is generally smaller than the total number of individuals within a population.

Evolutionary history: Past processes (**gene flow**, genetic drift [see below], mutation, selection) which have shaped the genetic variation within a population.

Evolutionary potential: The capacity of a population to evolve and respond to changes in the home-site environment.

Gene flow: The dispersal of genetic material in space and time from one individual to another or one population to another. For plants this is achieved by the dispersal of seed and pollen.

Genetic bottleneck: A rapid reduction in genetic diversity that can occur when population size is reduced.

Genetic diversity: The array of variable genetic characteristics or genotypes present within a population or species. Often measured with molecular markers. Genetic diversity arises through evolutionary processes, including mutation, **genetic drift**, **natural selection**, and migration (e.g. **gene flow**).

Genetic drift: In small populations some **alleles** or genotypes may become reduced in frequency or disappear by random chance if they are not passed on to further generations.

Genetic integrity: The maintenance of genetic diversity and evolutionary history of a population or species.

Genetic swamping: Rapid increase in frequency of an introduced genotype (or introduced **allele**) that might lead to replacement of local genotypes; caused by a numerical and/or fitness advantage.

Germplasm: Living tissue from which new plants can be grown e.g. seed, cuttings.

Heterosis: The improved health of hybrid offspring relative to the parents. Also referred to as hybrid vigour.

Home-site advantage: Fitness advantage of local genotypes (ecotypes) relative to introduced genotypes (ecotypes).

Hybridisation: The exchange of genes (or **alleles**) among different species (interspecific hybridisation) or populations (intraspecific hybridisation).

Inbreeding: Mating between related individuals with similar genetic makeup. This can result in inbreeding depression (see **Inbreeding depression**).

Inbreeding depression: Reduced fitness (i.e. growth and survival) due to mating among related individuals. May be of concern in small populations.

Local adaptation: A population which is fitter when grown in its own environment compared to other populations of the same species, but shows reduced fitness when the same population is grown in a non-local or foreign environment.

Local provenance: The geographic location of seed collected from individuals within a population in close proximity to the site being restored. The definition and extent of a local provenance can vary based on geographic, environmental, or genetic information.

Maladaptation: A trait that is more harmful than helpful, resulting in reduced fitness. Individuals well adapted to one habitat may be maladapted to a different habitat.

Mating system: Describes the type of mating for a plant species. Generally, self-fertilisation (pollen donor and recipient is the same individual), outbreeding or outcrossing (with pollen from a different individual) or mixed mating combining outcrossing and selfing in varied proportions among species and among populations.

Microsatellites: A form of molecular marker that identifies short repeated regions of DNA, which vary in size in different individuals. A widely used neutral molecular marker which is assumed to be not under natural selection.

Molecular marker: A fragment of DNA that is associated with a certain location within the genome. Many widely used molecular markers measure neutral genetic diversity.

Natural selection: A process whereby fit individuals which are better adapted to the environment pass on their genetic variation to their offspring.

Outbreeding depression: Reduction in mean population fitness relative to mid-parents, resulting from breeding between genetically distinct individuals or in extreme cases different species.

Phenotypic trait: An observed and measurable trait (such as height) that is an expression of genes. The combination of phenotypic traits (e.g. height, leaf length, etc.) expressed in a given environment represents a plants phenotype.

Plasticity: Reversible phenotypic changes with no changes to the DNA, such as changes in morphology or physiology, as a response to a change in the environment conditions.

Provenance: The geographic location of seed collected off individuals within a population.

Population: A group of interbreeding individuals that belong to the same species from a location.

Quantitative genetic traits: Quantitative traits show a range of variation influenced by both environmental and genetic factors.

Self-compatible mating system: A flower can be fertilised by pollen from the same individual and produce viable seed (opposite to self-incompatible).

Single nucleotide mutation: A single nucleotide base (e.g. A to T or G to C) change in the DNA sequence.

Single nucleotide polymorphism: When the mutation at a single nucleotide (see **single nucleotide mutation**) leads to a multiple variants / alleles that differ at a single base pair.

Translocation: The active movement of germplasm from one location to another, e.g. across geographic or environmental space.

Online resources

Atlas of Living Australia (ALA, https://spatial.ala.org.au/), shows species distribution, but keep in mind that some occurrence records might be miss-identifications or obvious ornamental plantings outside the known distribution range of the species. Similarly, contemporary and future climate data can be freely downloaded from various databases, including TERN (https://portal.tern.org.au/#/3da654fc), WorldClim (https://worldclim.org/data/index.html), and CHELSA (http://chelsa-climate.org/).

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