Conservation genomics of *Pimelea spicata* (Spiked Rice-flower) in support of management and translocation activities.

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FINAL REPORT

The Royal Botanic Gardens & Domain Trust

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Contents

EXECUTIVE SUMMARY

The genetic diversity of populations across the known distribution of *Pimelea spicata* was measured using high quality genome scans. Surprisingly, the most northern and the most southern sites of *P. spicata* are genetically most similar, and are differentiated from the rest of *P. spicata* populations that exist on the Cumberland Plain. There is little between-population genetic connectivity and as a result, individuals within populations tend to be similar to each other but different from those at other sites. The Western Sydney International Airport (WSI) site is the largest population and displays the highest genetic diversity of *P. spicata* among all tested sites. Two genetically distinguishable groups are present within the site at separate locations. The existing *ex situ* WSI collection at the Australian Botanic Gardens at Mt Annan (ABGMA) partially represents the diversity of one of these two groups and mostly contains unique genets (except for a small selection of replicated individuals). Genomic data was used to identify the propagules required to assemble a nursery population to be used in future translocation work. Assuring maximum levels of genetic diversity in a translocated population increases fitness by reducing the risk of inbreeding and increasing the adaptive potential to environmental change and other pressures. We estimated the necessary combinations of propagules to ensure the establishment of suitably evolutionary resilient translocated populations of various sizes. Our Environmental Niche Models suggest *P. spicata* has very little environmental suitability beyond its currently known distribution, and under future climatic projections suitable environments might be further constrained to the southern Cumberland Plain.

1. INTRODUCTION

1.1 Background

Pimelea spicata R.Br. (Thymelaeaceae), or 'Spiked Rice-flower', is an endangered subshrub from dry sclerophyll woodland of the Sydney Basin. It forms a lignotuber with a large tap-root (up to 18 cm by 24 mm in diameter) from which coppices numerous creeping, sometimes caducous, wiry shoots. Small compressed racemose inflorescences of pink to white flowers are produced at the terminus of these shoots, and flowers develop into a dry, indehiscent fruit with one seed. Poor resolution in the recent molecular phylogeny of *Pimelea* (Foster *et al.* 2017) makes it impossible to estimate the closest allied species to *P. spicata*. However, the few species of *Pimelea* that share its distinctive habit are recovered elsewhere in the phylogeny, showing that the species concept is unmistakeable.

The conservation listing for *Pimelea spicata* is endangered on both the NSW *Biodiversity Conservation Act 2016* and the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*. This species is threatened with extinction due to its highly fragmented and restricted distribution, which consists of up to 30 populations within the Cumberland Plain and eight others recorded in a disjunct area in the Illawarra coastal region (Willis *et al.* 2003). Remnant populations mostly exist in close proximity to developed areas which places them at high risk from weed competition and herbicide use during weed-control (Matarczyk *et al.* 2003).

Little is known about the reproduction and recruitment of *P. spicata*, consequently efforts towards securing genetically viable populations pose a challenge. Flowering time is inconsistent, flowering phenology and pollinators are unknown, and no experimental data have been acquired about whether the species is autogamous. In addition to the perceptible habitat loss in the Sydney area, some evidence suggests that the principal negative impacts threatening the species' survival are low recruitment rates (Dixon *et al.* 1995, Offord *et al.* 2009). However, the clandestine growth form and dormancy promoted by the lignotuber make tracking recruitment extremely difficult since seedlings may be difficult to distinguish from plants breaking dormancy, and delicate taproots impede age determinations. Very large lignotubers have been measured on some individuals (Matarczyk 1999), suggesting that *P. spicata* might reach considerably old age.

Loss of genetic diversity can reduce the genetic health of an organism, and as a result, increase the risk of local extinction. Genetic health is the concept used to explain population fitness and limitations to long-term adaptive potential to climatic and environmental changes.

Pimelea spicata has most likely suffered losses in genetic diversity, in addition to disrupted gene flow through the immense habitat fragmentation recently incurred throughout the Cumberland Plain and adjacent areas. Unfortunately, the state of genetic diversity across the species –past and present– is unknown, which severely compromises the ability to assess the health of the species, potentially limiting the efficacy of conservation action. Making matters worse, the resprouting habit of *P. spicata* compromises our ability to accurately assess recruitment and demography. These problems implicate that management actions at the population scale require better understanding of genetic health species-wide, and consequently a distribution-wide genetic study is essential to long-term the management of any *P. spicata* population.

One of the largest known populations of *P. spicata* is within the suburb of Badgery's Creek, which is the location of the new Western Sydney Airport. Removal of all plants during contruction in 2020 has been confirmed as a result of this population's placement within the Western Sydney International Airport (WSI) development site. As part of the Biodiversity Offset Delivery Plan (BODP) for the airport site, the Royal Botanic Gardens & Domain Trust (RBG&DT) was contracted by GHD on behalf of the Department of Infrastructure, Regional Development and Cities to deliver the Threatened Flora Propagation Program (TFPP) for *P. spicata*. The main objective of this program is to generate an *ex situ* collection of *P. spicata* from the Badgery's Creek population that can subsequently be used in research and restoration programs. An assessment of genetic diversity across the species was sought to equip the species with improved evolutionary resilience in the face of anthropogenic effects, and hence to help its survival. To achieve this, the RBG&DT investigated genetic diversity (qualitatively and quantitatively) and genetic health across the species, and used this information to define the optimal proportion of genotypes that are key to building and maintaining genetically resilient translocated populations.

1.2 Aims and objective of the conservation genomics study of *Pimelea spicata*

In order to support the long-term management and conservation of *Pimelea spicata*, the conservation genomics study had the following aims:

1. Describe genetic health, population structure and genetic diversity across the known distribution of *P. spicata*.
2. Describe the fine scale genetic health (i.e. level of genetic diversity) of the species at the Western Sydney International Airport (WSI) site.
3. Compare and determine diversity and kinship among *in situ* and *ex situ* plants (grown at the Australian National Botanic Gardens, Mt Annan) to elucidate how representative the current *ex situ* collection is.
4. Determine optimal selection of individuals across a number of translocation scenarios.
5. Investigate possible associations between genetic and geographic/environmental diversity, including differences between Illawarra and Cumberland Plain populations.

2. METHODS:

2.1 Sampling

The majority of sampling was undertaken by the Royal Botanic Gardens & Domains Trust (RBG&DT). A total 282 *Pimelea spicata* individuals were sampled across the extent of the known range, including populations north of the Hawkesbury river and populations in the Illawarra region (Fig. 1). The samples included 20 individuals from the preliminary *ex situ* collection of the Western Sydney International Airport (WSI) site currently in propagation at the Australian Botanic Garden, Mt Annan (ABGMA). Because precise geographic location information for each of the propagated individual in the collection was not available, we selected individuals that were spaced apart to avoid sampling multiple ramets (clones) of a genet.

At each *P. spicata* site (Fig. 1), a minimum of six individuals were collected at a minimum of ten metres apart where possible, and in such a manner to maximise the capture of diversity evenly across all populations, accounting for a variety of genetic barriers (e.g. creeklines). The populations that were selected varied in size, from six to several thousand individuals. The largest populations (WSI, Camden Golf Course, and Prospect Nature Reserve) were therefore sampled more intensively to increase the likelihood of capturing an accurate estimate of the true breadth of genetic diversity. Since the WSI site was a focus of this report, a detailed sample distribution is provided (see results).

Separate sampling methodologies were used for differently sized populations:

i. Population with several thousands of individuals: transects were made to investigate how is genetic diversity distributed within those large, continuous populations (see Table 1). The key point in this methodology is a larger collection of samples per population (12 -24 individuals throughout the extent of the population).
ii. Population with up to several hundreds of individuals: six individuals were collected as evenly as possible across the distribution of the population, to assess whether diversity is present and how much of it.

2.2 DNA extraction and sequencing

All samples were sent to Diversity Arrays Technology (DArT) Pty Ltd in Canberra for DNA extraction and genotype-by-sequencing analysis (referred to as DArTseq analysis) using the documented in-house procedure. DNA was extracted from each sample using the Plant DNA Extraction Protocol for DArT.

2.3 Data analysis

2.3a Quality screening and control of Single Nucleotide Polymorphism data:

Single nucleotide polymorphisms data (SNPs) was checked for quality using the filtering scripts implemented by an in-house designed package called RRtools package v1.0 (as described in Rossetto *et al.* 2019) in the open source program, R (version 3.3.0, R Core Development Team 2013). Loci that did not pass standardised quality thresholds were removed from the data and were not used in downstream analysis. To ensure that only the higher quality DArTseq markers were used for analyses, all SNPs with a reproducibility (proportion of replicate assay pairs for which the marker score is consistent) of less than 96% and which had more than 30% missing data were excluded from the dataset.

2.3b Principal coordinate analyses

Adegenet 2.1.1 package in R (version 3.3.0, R Core Development Team) was used to perform a Principal Component Analysis (PCA) to better understand relationships between individuals and populations. This method of PCA derives an ordination based on Euclidean transformed dissimilarity matrix of the data.

2.3c Genetic structure

LEA v2.4.0 (an R Package for Landscape and Ecological Association Studies) was implemented to perform a test of genetic structure. This statistical method used estimates ancestry coefficients from large genotypic matrices and evaluates the number of ancestral populations. LEA uses the snmf function (Sparse Non-Negative Matrix Factorization algorithms) to estimate individual admixture coefficients from the genotype matrix. A measure of fit (i.e. the entropy criterion) is evaluated between the statistical model and data

and is used to choose the best number of ancestral populations that explain the data. We examined the minimum cross-entropy for up to K = 8, wherein we selected the optimal number of ancestral populations (K) based on the post-stabilisation of the steepest decline in cross-entropy values.

2.3d Phylogenetic analyses

Phylogenetic analysis, unlike distance-based methods, is important for understanding the relationships between a group of organisms. A phylogenetic tree infers relationships from an evolutionary history, rather than through observed genetic or phenetic similarities. We constructed a coalescent-based phylogenetic tree to estimate population relationships for *P. spicata*. Our sample set was informed through the results of our kinship analysis: all genets of *P. spicata* were sampled. We conducted the coalescent analysis of our sample set with the SVDquartets package ver. 1 (Chifman *et al.* 2014) implemented in the PAUP software v4.0a (Swofford 2002). The advantage of this program is that it is designed to accept SNP data and it produces robust phylogenetic results (see Chou *et al.* 2015 for a critical review of this program). Settings included evaluating 100,000 quartets, 1000 bootstrap replicates, and the multispecies coalescent tree model selected. We examined results of all analyses using at least three independent runs for multi-species coalescent analysis by allocating samples within their respective populations.

2.3e Population genetic diversity measures

In order to evaluate F-statistics and population-level measures of diversity, the RRtools v1.0 package was used. The outputs for the F-statistics is in the form of a matrix of spatial distances between the populations, and the diversity measures are the expected (H_e) and observed heterozygosity (H_o) and the inbreeding coefficient (F_{is}) across each population.

2.3f Kinship

Genetic similarity between individuals located at the same site and corresponding cultivated material was estimated using the unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering method as implemented in the phanghorn package v2.4.0 in R. Kinship (relatedness) measurements were used in assessing the degree of clonality across the *in situ* plants of *P. spicata*, and identify genets and ramets of *P. spicata* in cultivation.

Pairwise kinship coefficient was estimated from the genotype data using an Identity-by-descent (IBD) analysis in SNPrelate package v1.17.1 in R. Distance matrices of pairwise kinship were generated for each *P. spicata* site based on observation from preliminary results from Principal component and network analysis that clonality occur within each site. The matrices were combined to generate a supermatrix that was drawn using the heatmap function from the Phytools package v0.6-60.

2.3g Optimal genetic diversity for translocation

If maximal genetic diversity (and hence greater expected fitness) is desired in a population created by future translocation efforts, an explicit proportion of genetically distinct individuals (i.e. genets determined from the UPGMA results in Table 1) will be required. Given the large number of genets at the Western Sydney International Airport site (both *in situ* and *ex situ* of WSI), we explored using the number of initial propagules and the target size for a translocated population (i.e. number of cuttings for any given number of starting propagules) for the translocation options. Optimisation of mixtures analyses were implemented on the package OptGenMix developed at the RBG&DT, Sydney, (Bragg *et al*. 2019). This package sought the optimal proportion of genets by evaluating the highest proportion of shared alleles among individuals for all combinations. The package recommends having multiple ramets (either cuttings or individuals with the same genet) for each selected genotype for two biologically meaningful reasons: 1) our estimate of genetic diversity is not always comprehensive, and therefore genetically similar individuals might represent important variation at any given allele; 2) survival of translocated individuals is not guaranteed, and having more cuttings from the same genet might buffer a complete loss of one genotype, at least to some extent.

2.3h Environmental niche modelling

Climate change is a major cause of biodiversity loss and is expected to be a key process resulting in species extinctions, and its effects will be most severe for endangered species. Therefore it is prudent to carefully select sites for translocation of *P. spicata* given that current habitat availability will shift in response to climate change. To further inform site selection process, environmental niche models were used to examine changes in the area of habitat availability for *P. spicata*. This was completed by acquiring an overall understanding of the environments associated with the species with MaxEnt modelling method implemented in

the R-package maxnet (Phillips *et al.* 2017; Phillips *et al.* 2017). Environmental data used in the niche modelling was assembled from several sources:

Current or observed climate was provided by the ANUClimate dataset (http://geonetworkrr9.nci.org.au/geonetwork/srv/eng/catalog.search#/metadata/f30e39a4-15dc-4038-a37f-7fd29744e46a) and monthly minimum and maximum surface temperature, and monthly rainfall were downloaded for the period 1983 to 2012 providing a 30-year climate baseline. We used these monthly data to compute the standard 19 bioclimatic variables. CSIRO data for soil composition (percent sand, silt and clay) and topographic information (slope, aspect, topographic position index and topographic wetness index) (https://data.csiro.au/dap/home?execution=e1s1) was downloaded and re-sampled to the same grid as the climate data. Climate data for past and future climate was computed using data from the Fifth Assessment Report of the Inter-governmental Panel on Climate change (IPCC) by applying the delta or anomaly method to current climate data. Data for several General Circulation Models/Earth System Models (GCMs/ESMs) were downloaded from the Climate Model Inter-comparison Project version 5 (CMIP5) website, but we selectively downloaded data only for models which were assessed as having skill in modelling climate conditions for the Australian region (Whetton *et al.* 2015).

The MaxEnt model fitted to current environmental conditions was projected onto environmental data for future climates under two scenarios for increases in greenhouse gases: Moderate increase (known as the Resource Concentration Pathway scenario 4.5 or RCP4.5), and extreme increase (RCP8.5). Future climate was computed for each RCP for two 30-year climate averages centered on 2050 and 2070.

The R-package dynRB (Junker *et al.* 2016) was then used to compute niche overlaps between genetically-defined groups of occurrence records for *P. spicata*. This allowed us to highlight differences or similarities in the environmental niches of pairs of groups. The three genetically-defined groups of occurrence records (South, Central and North) were for the degree of environmental niche overlap at the known occurrence locations under current (observed climate) and predicted climate at these locations predicted for 2050 and 2070 under moderate (RCP4.5) and extreme (RCP8.5) climate change scenarios.

3. RESULTS AND INTERPRETATION

3.1 Summary

We report results based on high-quality genome scans from DArTseq that enabled quantification of genetic diversity and relatedness between and within populations, assessment of kinship and an estimation of associative patterns between genetic and geographic structure. Additionally, environmental niche models were investigated to examine potential future shifts in the availability of putatively suitable habitat.

The significant findings for *P. spicata* are:

- Genetic variability exists within all sites (with the exception of Wilberforce);
- Gene flow between sites is lower than gene flow within sites, despite the small area covered by the species (max. distance of 100 km);
- The northern and southern *P. spicata* sites outside the Cumberland Plain habitat are more closely related to each other than the rest of the species;
- The Western Sydney International Airport (WSI) site has the highest genetic diversity, and consists of two genetically distinct groups;
- The *ex situ* WSI collection of *P. spicata* consists mostly of unique genets (except for a small selection of replicated individuals) but is not entirely representative of the genetic diversity present at the site;
- Very little environmental suitability is projected beyond the currently known distribution, and the future availability of suitable habitat is predicted only in the southern Cumberland Plain
- Translocation scenarios based on the empirical evolutionary information on *P. spicata* are provided.

3.2 Genetic health and population structure across *Pimelea spicata*.

A Recovery Plan prepared for the endangered shrub species *Pimelea spicata* (NSW Department of Environment and Conservation 2005) described the species as often occurring adjacent to urban areas, and as impacted by urban development. It is therefore important to consider evolutionary resilience in long-term management strategies for *P. spicata*. The genomic signature we obtained from 41,000 genome-wide markers (SNPs), for

282 samples representing 16 *P. spicata* sites shows that the species is still genetically variable within and between sites (Fig. 1, 2, 3, 4), which is a positive starting point for long-term planning.

Our empirical data determines the extent of clonality, kinship and genetic diversity within all *P. spicata* sites. Table 1 lists the number of genets present at each site based on the sampled cohort, the number of genets that have multiple ramets, and the number of individuals observed at each site partitioned into various heights as potential surrogates for parents and clones. Each *P. spicata* site consists of at least one distinct genet (i.e. not found in other sites), and the extent of clonality varies from site to site but is generally low (Table 1, 2). This of course is also dependent on the sampling strategy, but empirical data suggests that generally, individuals sampled a few meters apart are unlikely to be clonal.

Genetic diversity was measured using expected and observed heterozygosities (Table 3). Most sites displayed moderate levels of heterozygosities, with the exception of Freemans Reach, Blackbutt Nature Reserve, Mt Warrigal and Wilberforce, which showed lower levels. The Wilberforce site consisted of the lowest genetic diversity since all six samples tested belonged to a single genet (Fig. 5). Genets with multiple ramets were also observed at Merrylands and at ABGMA (Table 2). Overall most populations show positive F_{is} values, suggesting the likelihood of some biparental inbreeding (as expected in circumstances of relatively limited gene flow) within most sites (Table 3). Nevertheless, overall kinship values are generally low and no genets are shared among sites, suggesting apomixis (clonal seed production) in *P. spicata* is unlikely.

The Principal Component Analysis (PCA) shows that most *P. spicata* sites are genetically differentiated, with the pair of sites at the most northern (Wilberforce, Freemans Reach) and southern (Blackbutt Nature Reserve and Mt Warrigal) range limit of *P. spicata* being the most distinct from the rest (Fig. 1b). Although separated by the Cumberland Plain, these same northern and southern sites are also more genetically similar to each other than to the rest of the sites. The PCA interpretation is supported by population structure analysis (Fig. 1a) and phylogenetic analysis (Fig. 2) in showing that the northern and southern sites are distinct from the rest. Comparing the environmental niches across the northern (includes Wilberforce, Freemans Reach), southern (includes Blackbutt Nature Reserve and Mt Warrigal sites) and central (Cumberland Plain) extent of *P. spicata* showed moderate niche overlap between the

northern and central distributions, with the environments in the south displaying a distinct climate regime from the rest of the species range (Fig. 6). This relationship remains the same under all projected changes in climate with some increase in the overlap between central and northern extent.

Pairwise F_{st} measures corroborate these findings, with Wilberforce, Freemans Reach, Blackbutt Nature Reserve and Mt Warrigal showing high F_{st} when compared to other sites supporting expectations from reduced gene flow (Fig. 3b). F_{st} results also show that among the remaining *P. spicata* sites gene flow decreases as geographic distance between them increases (isolation by distance; Fig. 3a). In other words, there is little between-population genetic connectivity and as a result, individuals within populations tend to be similar to each other but different from those at other sites.

3.3 Genetic health of *Pimelea spicata* at the Western Sydney International Airport Site.

One of the largest populations of *P. spicata* plants known is at the Western Sydney International Airport (WSI) site where development is underway. Consequently, genetic sampling at the WSI site was much more intensive than at other sites and was complemented by samples of the relevant *ex situ* collection at ABGMA.

Our kinship analysis on all WSI individuals indicates that all *in situ* WSI individuals display unique genotypes, and almost all *ex situ* WSI individuals are also unique – with the exception of seven individuals belonging to genets with multiple ramets (Fig. 5, Table 2). This is probably the result of sampling and propagation of multiple cuttings from a single genet by ABGMA. Overall, as suggested in the previous section, the WSI population is very diverse with high levels of heterozygosity, and relatively low kinship suggesting that within population gene flow occurs efficiently (Table 3).

Interestingly the population-specific PCA (Fig. 4) suggests the presence of microsite differentiation within the WSI site, with individuals along The Northern Road and in the vicinity of a creek being somewhat distinct. This is in agreement with the patterns of overall biparental inbreeding and the geographic distance-based divergences (to which habitat

fragmentation most likely contributes) observed across the whole distribution of the species. The PCA also suggests that the *ex situ* individuals (black dots in Fig. 4b) display genetic variation, but currently do not completely represent the breadth of diversity present at the WSI site.

Finally, overall the sites that are most closely genetically related to the WSI are represented within a clade that includes ABGMA and the Camden Golf Course which are also relatively large and close to each other (Fig. 2). Based on environmental niche modelling (ENM) data, we predict southern parts of the Cumberland Plain which includes ABGMA, Camden Golf Course and Minto to retain suitable environments for *P. spicata* under available climate change scenarios (Fig. 7b, c, d, e). However, there are limits to the information that can be provided about the Cumberland Plain as the narrow range of *P. spicata* limits the predictive power of the models.

3.4 Projected estimates for potential translocations of *Pimelea spicata*.

Genomic data was also used to identify the propagules required to assemble a nursery population to be used in future translocation work. Assuring maximum levels of genetic diversity in a translocated population increases fitness by reducing the risk of inbreeding and increasing the adaptive potential to environmental change and other pressures. Consequently, we estimated the necessary combinations of *in situ* and *ex situ* propagules to ensure the establishment of suitably evolutionary resilient translocated populations of various sizes (Table 4).

We first tested the diversity potential of translocation populations through simulations. The simulations confirmed that selecting individuals by optimising on the basis of genomic data was more effective at capturing higher genetic diversity than using a random sampling approach (Fig. 8). The simulations also identified that a translocated population of 250 individuals can capture 90% or more of the diversity currently found within WSI (based on simulations that predicted the genetic diversity remaining after 10 generations of genetic drift; Fig. 8). We could then use that information to model how many genetically distinct individuals need to be propagated for inclusion within such a population (using an algorithm that selects individuals that maximize genetic diversity; Fig. 9).

Based on the simulation results and the empirical genomic data, Table 4 provides a range of scenarios on what individuals to propagate and include in a translocation project. It should be noted that the numbers we report for each of the population size are not a proportion, and a similar analysis for a larger population size might not return precisely the same result as would multiplying results in Table 4. This is exemplified by the additional translocation scenario (Table 5) involving a translocated population of 100 individuals (capturing 85% or more diversity currently found within WSI). New numbers can and will be provided as needed if circumstances change (for example if survival of the targeted propagules is different from initial objectives).

4. CONCLUSIONS AND IMPLICATIONS

This project highlights the following results:

- Most *Pimelea spicata* sites display genetic variability, with the greatest genetic diversity found at the WSI site;
- Overall levels of differentiation between sites is relatively consistent and associated with geographic distance between the sites, with the exception of the most northern and southern sites which are more genetically differentiated;
- *P. spicata* can rely on little environmental suitability outside its currently known distribution, and future climatic projections predict the persistence of suitable habitat only within the southern Cumberland Plain;
- The *ex situ* WSI collection currently held at ABGMA based on a random sampling approach is diverse but does not fully represent the genomic diversity remaining at the site.

A range of translocation scenarios ensuring the maintenance of maximum diversity are proposed, these recommend:

- the use within translocated populations of 256 (or greater) individual plants;
- the preferential use of genomically selected individuals;
- individuals to use to maximise long-term evolutionary resilience as based on initial propagation target of 16, 32, or 64 individuals.

5. FIGURES AND TABLES

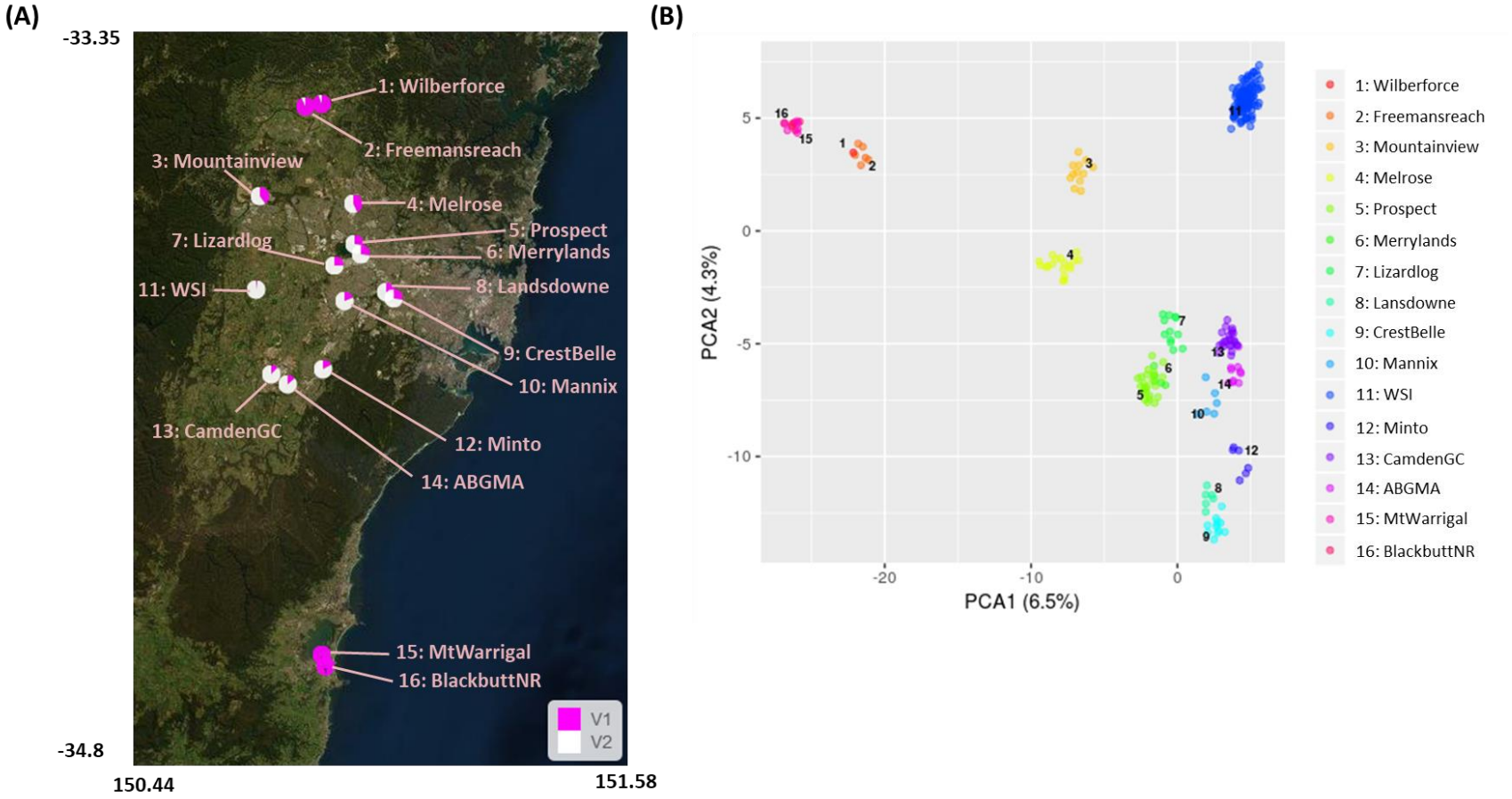


Figure 1: Study sites, population structure analysis (A) and principal component analysis (B) of *Pimelea spicata* at the Cumberlain Plain and the Illawarra region, New South Wales. Each pie chart in the map (A) indicates a study site, with the exception of samples obtained from the Australian Botanic Garden at Mt Annan (ABGMA). The samples were separated into *ex situ* collection from Western Sydney Airport cultivated at ABGMA ("WSI_*Ex situ*") and *in situ* collection at ABGMA ("ABGMA"). The population structure and PCA analyses were generated from Single Nucleotide Polymorphism data for 200 specimens of *P. spicata* with unique genets (determined from kinship analysis as detailed in methods). The pie charts show the averaged ratios of assigned ancestry within each site from population structure analysis involving K=2 ancestry populations.

Table 1: *Pimelea spicata* study site details and number of genets at each site. Western Sydney International Airport (WSI) consists of an *in situ* (N=100) and *ex situ* (N=20) collection. Three genets with multiple ramets were observed among the *ex situ* samples, see Table 2 for details.

Site	Site description	Latitude	Longitude	N sampled individuals	N genets with multiple ramets	Maximum distance between samples (m)	Total N genets
Wilberforce	Wilberforce cemetary	-33.5541	150.8436	6	1	13.7	1
Freemansreach	Freemans Reach	-33.5597	150.8053	6	0	39.5	6
Mountainview	Mountain View Reserve	-33.7212	150.7015	12	0	1262.7	12
Melrose	Melrose Park	-33.7358	150.8988	18	0	2585	18
Prospect	Prospect Nature Reserve	-33.8094	150.9138	24	0	1266.3	24
Merrylands	Merrylands Nature Reserve	-33.8264	150.9286	6	1	13.4	5
Lizardlog	Lizard Log	-33.849	150.87	12	0	1669.3	12
WSI	Western Sydney International Airport	-33.8903	150.6987	120	3	504.7	117
Lansdowne	Lansdowne Mirambeena Reserve	-33.8944	150.9768	6	0	552.8	6
CrestBelle	Crest Belleview Reserve, Bankstown	-33.9076	150.9827	12	0	1471.2	12
Mannix	Mannix Park	-33.9109	150.8922	6	0	147.2	6
Minto	Corner of Pembroke Road and Ben Lomond Road, Minto	-34.0335	150.8458	6	0	18	6
CamdenGC	Camden Golf Course	-34.0445	150.7324	24	0	558.4	24
ABGMA	Australian Botanic Garden, Mt Annan	-34.0628	150.7677	20	2	NA	10
MtWarrigal	Mount Warrigal	-34.548	150.8424	6	0	46.6	6
BlackbuttNR	Blackbutt Nature Reserve	-34.5688	150.85	6	0	269.5	6
			Total study samples	282		**Total unique genets**	271

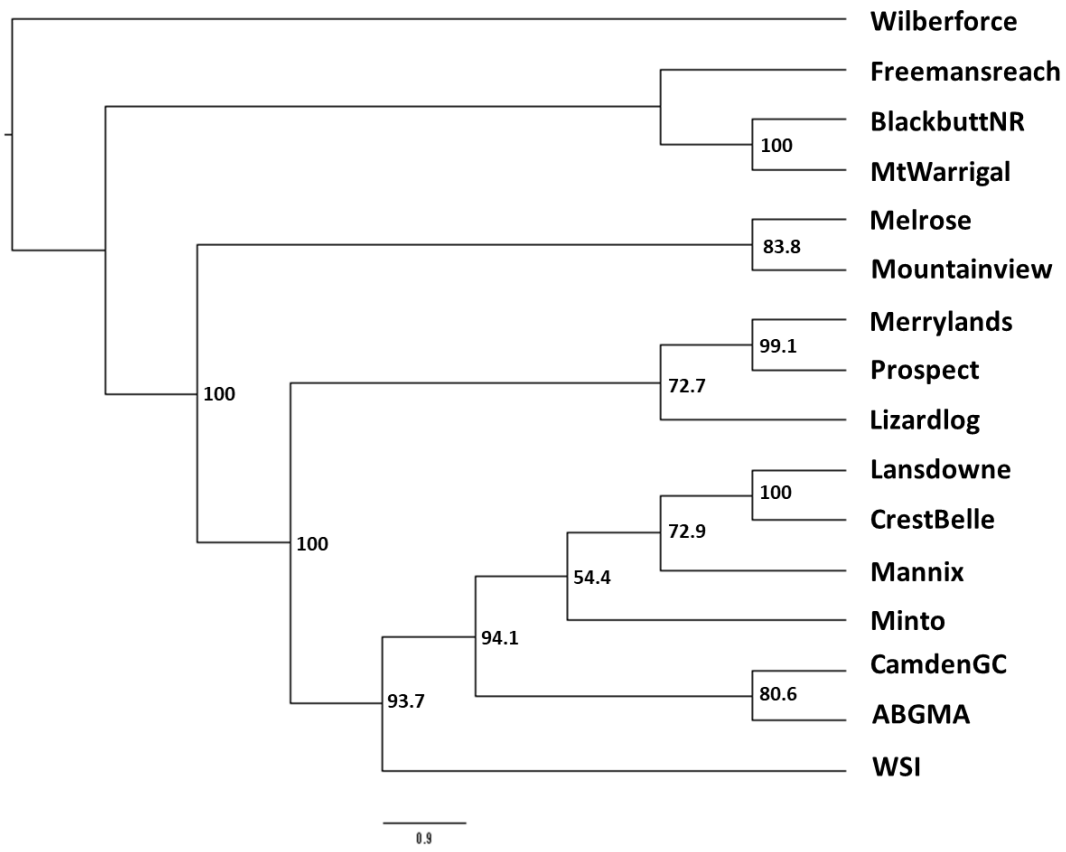
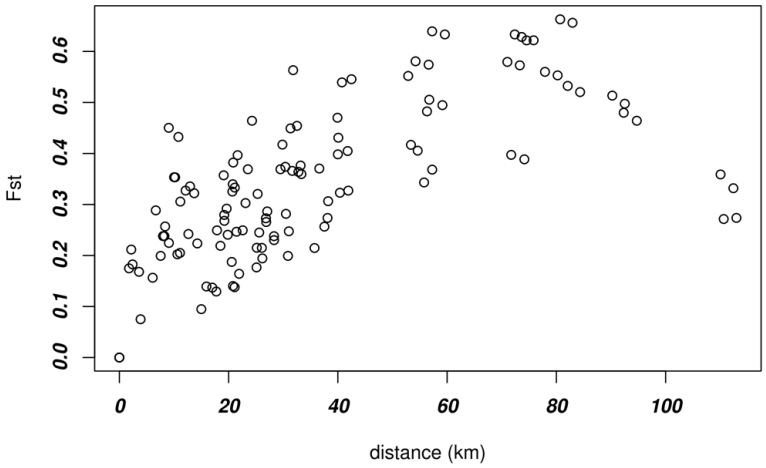


Figure 2: Phylogenetic coalescent tree produced from SVDquartets analysis of *Pimelea spicata* shows the samples across the distribution of species form a well-supported monophyletic clade. Each terminal on the tree represents a site of *P. spicata* except for "WSI" which consists of *in situ* (WSI) and *ex situ* samples (WSI_Ex situ) from Western Sydney International Airport. Bootstrap support values above 50% are placed above branches. The total weight of incompatible quartets = 197 (10.8%), and total weight of compatible quartets = 1623 (89.2%).

(A)



(B)

	Wilberforce	Freemansreach	Mountainview	Melrose	Prospect	Merrylands	Lizardlog	WSI	Lansdowne	Crestbelle	Mannix	Minto	CamdenGC	ABGMA	Mt_Warrigal	BlackbuttNR
Wilberforce	NA															
Freemansreach	0.168	NA														
Mountainview	0.298	0.338	NA													
Melrose	0.378	0.409	0.256	NA												
Prospect	0.410	0.463	0.264	0.283	NA											
Merrylands	0.449	0.563	0.381	0.398	0.244	NA										
Lizardlog	0.390	0.461	0.249	0.262	0.184	0.312	NA									
WSA	0.326	0.325	0.139	0.191	0.161	0.249	0.140	NA								
Lansdowne	0.431	0.539	0.369	0.390	0.359	0.450	0.337	0.216	NA							
CrestBelle	0.441	0.541	0.365	0.398	0.363	0.454	0.338	0.223	0.177	NA						
Mannix	0.398	0.470	0.276	0.301	0.237	0.353	0.210	0.137	0.238	0.237	NA					
Minto	0.417	0.552	0.374	0.400	0.356	0.464	0.329	0.220	0.292	0.291	0.224	NA				
CamdenGC	0.376	0.444	0.227	0.268	0.221	0.336	0.191	0.090	0.256	0.253	0.151	0.220	NA			
ABGMA	0.387	0.484	0.285	0.326	0.282	0.389	0.258	0.132	0.290	0.288	0.198	0.241	0.084	NA		
MtWarrigal	0.271	0.359	0.481	0.528	0.578	0.663	0.571	0.401	0.628	0.632	0.579	0.639	0.550	0.586	NA	
BlackbuttNR	0.274	0.332	0.467	0.517	0.570	0.656	0.561	0.393	0.622	0.625	0.573	0.633	0.547	0.582	0.183	NA

Figure 3: Isolation by distance graph (A) and Pairwise matrices of F_{st} values derived from single nucleotide polymorphism (SNP) data for *Pimelea spicata* sites (B). (A) The F_{st} values increase corresponding with distance (meters) between any two given individuals, demonstrating the trend of isolation by distance. Results from a Mantel test using 9999 permutations provided an R value of 0.626 that is statistically significant ($p < 0.001$). **(B)** Heat map colours, from green to red, are indicative of lowest to highest F_{st} values, respectively.

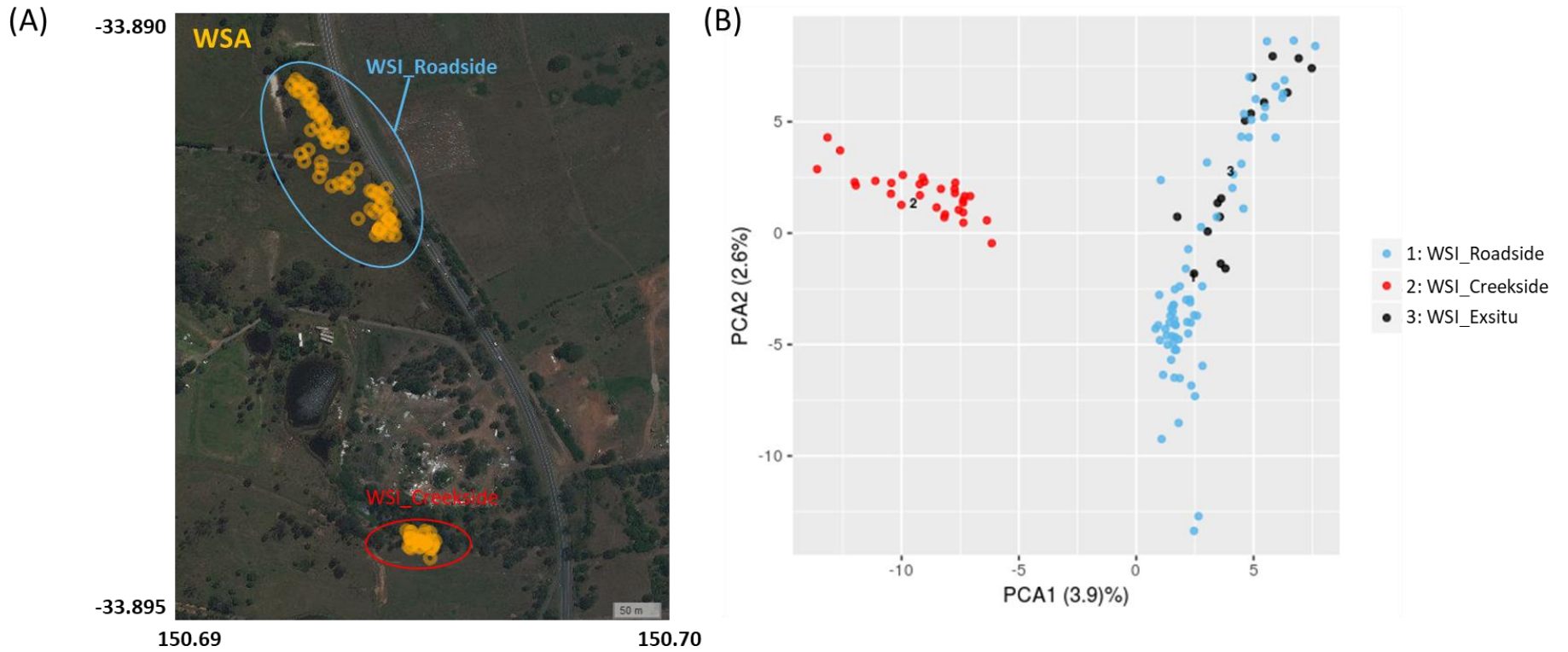


Figure 4: Detailed sampling (A) and principal component analysis (B) of *Pimelea spicata* from Western Sydney International Airport: In (A), each circle represents a sampled individual, and individuals were collected along The Northern Road (labelled as "WSI_Roadside") and in the vicinity of a creek (labelled as WSI_Creekside). The PCA shows the genetic relationship among the *in situ* and *ex situ* collection of Western Sydney International (WSI) Airport.

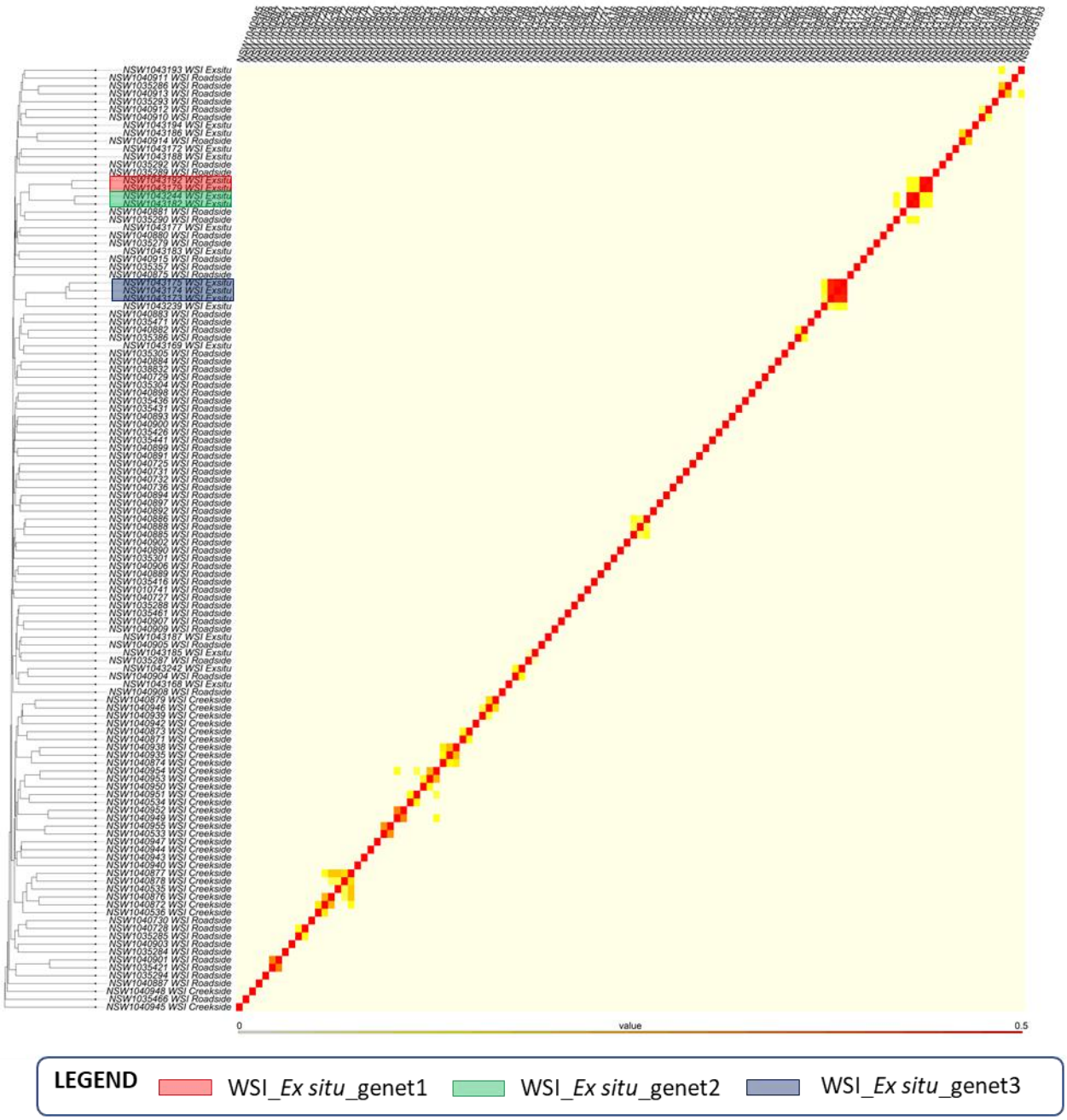


Figure 5: Composite UPGMA tree/Kinship heatmap analysed from Single Nucleotide Polymorphism loci for 120 *in situ* and *ex situ* specimens of *Pimelea spicata* from the Western Sydney International Airport (WSI) site. Heatmap shows all *in situ* WSI individuals are unique genets, and ex *situ* WSI specimens from the Australian Botanic Gardens, Mt Annan (ABGMA) had three genets with multiple ramets among 14 unique genets (see Table 2). Heatmap displays kinship estimated for each pair of samples in WSI: RED colouration corresponding to the highest kinship (0.4 or greater = clone) and white corresponding to the lowest kinship (0). The descending red diagonal on the graph is therefore the result of an individual matched with itself. The UPGMA tree shows individuals from ABGMA are clustered with individuals from WSI_Roadside rather than individuals from WSI_Creekside (refer to Figure 4 for map of WSI).

Table 2: A list *Pimelea spicata* samples belonging to genets with multiple ramets. E.g., NSW1043179 and NSW1043192 are ramets of the same genet type "WSI_*Ex situ*_genet1".

Site	Sample	Genet type
Wilberforce	NSW1040541	Wilberforce_genet1
	NSW1040538	Wilberforce_genet1
	NSW1040455	Wilberforce_genet1
	NSW1040544	Wilberforce_genet1
	NSW1040537	Wilberforce_genet1
	NSW1040454	Wilberforce_genet1
Merrylands	NSW1040917	Merrylands_genet1
	NSW1040922	Merrylands_genet1
ABGMA	NSW1043197	ABGMA_genet1
	NSW1043196	ABGMA_genet1
	NSW1043201	ABGMA_genet2
	NSW1043199	ABGMA_genet2
WSI_ *Ex situ*	NSW1043179	WSI_*Ex situ* genet1
	NSW1043192	WSI_*Ex situ* genet1
	NSW1043182	WSI_*Ex situ* genet2
	NSW1043244	WSI_*Ex situ* genet2
	NSW1043173	WSI_*Ex situ* genet3
	NSW1043174	WSI_*Ex situ* genet3
	NSW1043175	WSI_*Ex situ* genet3

Table 3: Observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}) and number of unique genets (N) identified at each site and the microsites at the Western Sydney International Airport (WSI) site.

Site	H_o	H_E	F_{IS}	N
Wilberforce	0.056	0.226	NA	1
Freemansreach	0.109	0.121	0.072	6
Mountainview	0.174	0.211	0.146	12
Melrose	0.145	0.198	0.223	18
Prospect	0.158	0.216	0.235	24
Merrylands	0.151	0.141	-0.074	5
Lizardlog	0.153	0.214	0.23	12
WSI	0.203	0.267	0.236	117
Lansdowne	0.133	0.166	0.15	6
CrestBelle	0.127	0.18	0.24	12
Mannix	0.177	0.205	0.101	6
Minto	0.157	0.167	0.048	6
CamdenGC	0.199	0.247	0.169	24
ABGMA	0.186	0.219	0.121	10
MtWarrigal	0.062	0.076	0.152	6
BlackbuttNR	0.066	0.081	0.14	6
Overall	0.174	0.282	0.38	271
WSI site				
WSI_Roadside	0.19	0.259	0.252	70
WSI_Creekside	0.182	0.232	0.255	30
WSI_Exsitu	0.082	0.142	0.158	17

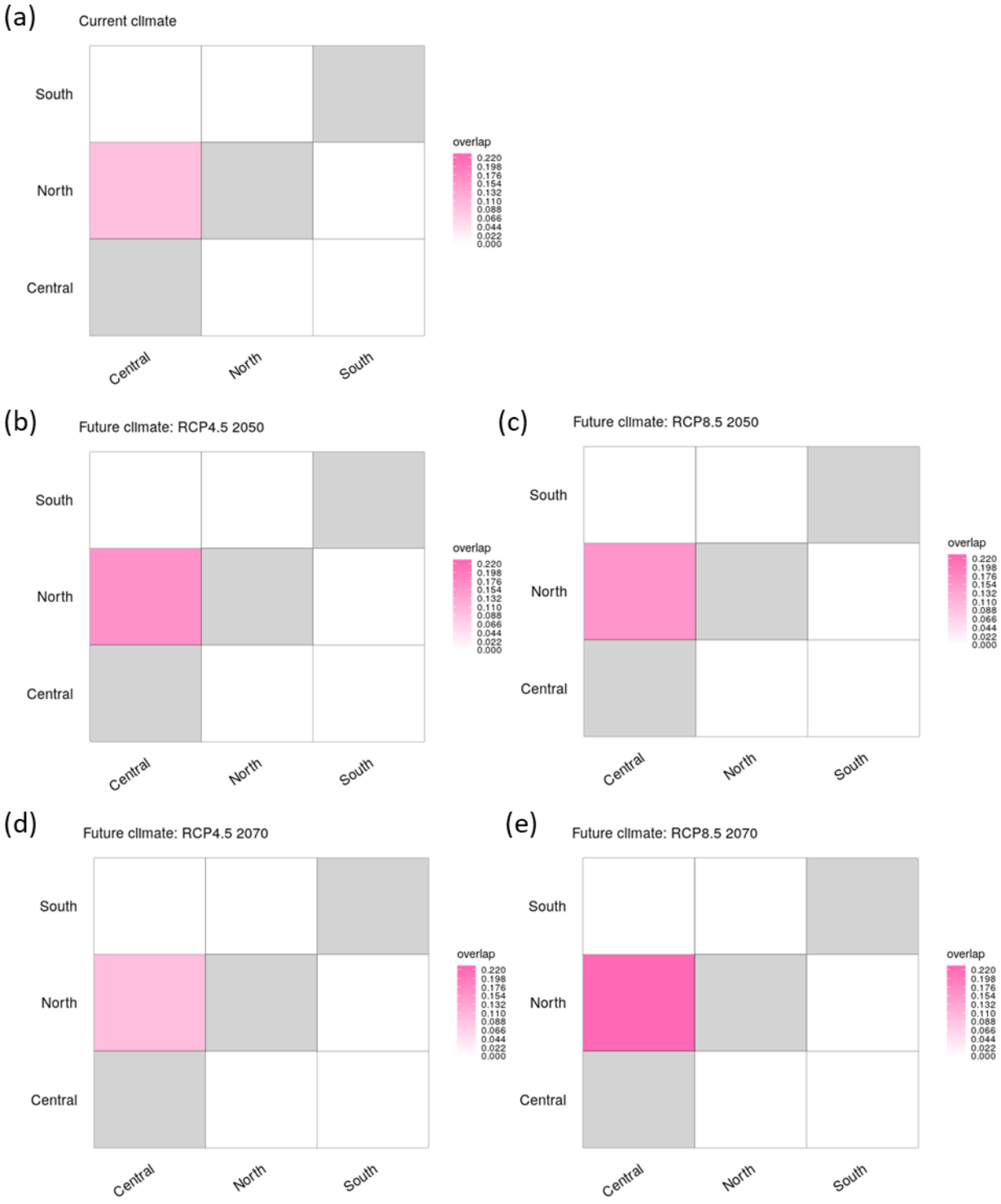


Figure 6: Niche overlap plots for the Northern (north of the Hawkesbury river near Windsor and Richmond), Southern (Illawarra area) and Central (Cumberland Plain) geographic extent of *Pimelea spicata* under the current (a) and future (2050 (b,c) and 2070 (d,e)) climatic conditions, with different future scenarios (RCP4.5, RCP8.5 respectively) provided. (see caption below)

Future climatic scenarios were generated based on different Representative Concentration Pathways or RCPs, with RCP 4.5 being more conservative than RCP 8.5 for projecting a lower temperature increase hence lesser impact from climate change.

There is a moderate degree of overlap between central and northern extent but both have distinctly different climate regimes from the southern extent. This relationship remains the same under all projected changes in climate with some increase in the overlap between central and northern extents.

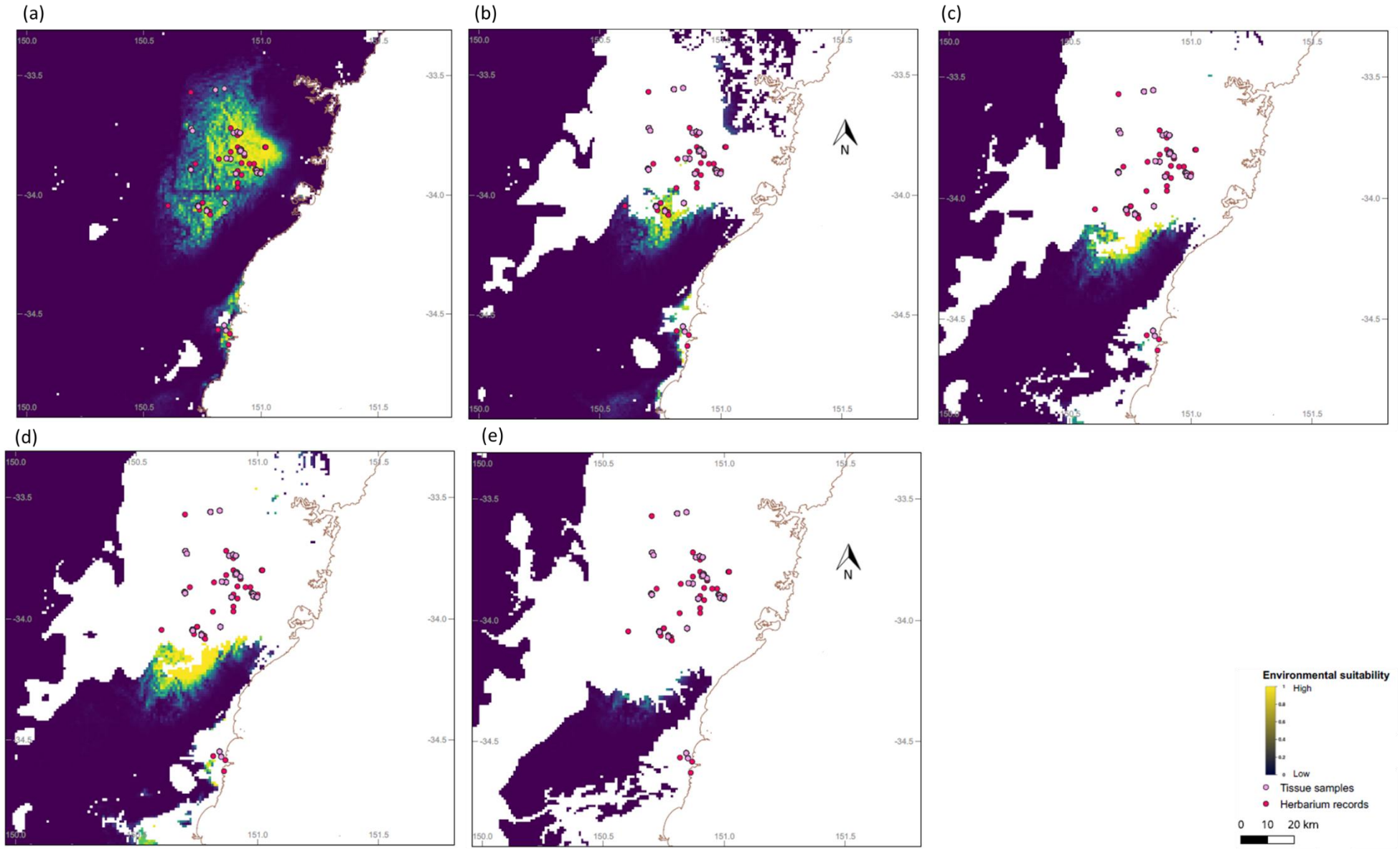


Figure 7: Environmental niche models for *Pimelea spicata* under current (a) and future (2050 (b,c) and 2070 (d,e)) climatic conditions, with different future climatic scenarios (RCP4.5 and RCP8.5 respectively) provided. (see caption below)

In each model, the area of suitable habitat (yellow – most favourable, dark blue – unfavourable) is presented. The areas in white (b, c, d, e) are where the model was attempting to make predictions using environmental variable values outside those presented during model fitting. By removing these areas of extrapolation it ensures that model interpretation is more rigorous and less prone to drawing false conclusions regarding environmental suitability because only values supported by the available data are presented. For example, the white areas in the future climatic models can be interpreted as having environmental conditions that are beyond those currently suitable for the species, and so it is not known whether it is suitable for the species.

The fitted niche models indicate *P. spicata* has very little environmental suitability beyond its currently known distribution. Under future climatic scenarios, suitable environments will be present in southern Cumberland Plain, in areas where species currently occurs (including the Minto, ABGMA and Camden Golf Course sites studied here; use BioNet Species Sightings Search: https://www.environment.nsw.gov.au/atlaspublicapp/UI_Modules/ATLAS_/atlasreport.aspx) or further south within the Wollondilly local government area (https://www.environment.nsw.gov.au/threatenedspecies/MapOfTheCumberlandPlain.htm;).

Table 4: Translocation options for the tagged individuals at the Western Sydney International Airport site to generate a propagation population of optimal genetic diversity.

This table lists options for producing a population of 256 propagules (based on the model presented in Figure 6) from various starting numbers of propagated individuals: 16, 32, 64 individuals and "Unrestricted" (an ideal case with no limits to the number of starting propagules). For each translocation option, the original individual to be propagated and the number of cuttings to be obtained from it to reach a total of 256 for an ideal translocated population is listed.

Each *in situ P. spicata* individual (with unique NSW number) has its own number tag (number was marked on a temporary tag using a permanent marker pen) and an embossed metal tag with a set of unique numbers/letter (e.g A311). GPS coordinate of each *in situ* individual is listed in the table.

Ex situ individuals were sampled from the ABGMA nursery and each have their own unique accession number.

	Translocation Options	Tag ID	If pick 16 individuals	If pick 32 individuals	If pick 64 individuals	Unrestricted	Latitude	Longitude
In situ	NSW1040727	31-A311	0	0	4	1	-33.8909	150.6989
In situ	NSW1010741	23-303	0	8	4	3	-33.8915	150.6993
In situ	NSW1040729	30-A310	0	0	0	0	33.89102	150.6989
In situ	NSW1040736	20-300	16	8	4	11	-33.8903	150.6986
In situ	NSW1040897	82-331	0	0	0	0	-33.8903	150.6987
In situ	NSW1035441	17-297	0	0	4	0	-33.8903	150.6987
In situ	NSW1040892	78-327	0	0	0	0	-33.8903	150.6986
In situ	NSW1040731	19-299	0	0	0	0	-33.8903	150.6986
In situ	NSW1040894	79-328	0	8	4	4	-33.8903	150.6986
In situ	NSW1035436	16-296	16	8	4	10	-33.8904	150.6988
In situ	NSW1040891	77-326	0	8	4	4	-33.8904	150.6987
In situ	NSW1040898	83-332	16	8	4	6	-33.8904	150.6988
In situ	NSW1040899	84-333	0	0	0	0	-33.8904	150.6988

	Translocation Options	Tag ID	If pick 16 individuals	If pick 32 individuals	If pick 64 individuals	Unrestricted	Latitude	Longitude
In situ	NSW1040900	85-334	16	8	4	9	-33.8905	150.6988
In situ	NSW1040893	76-325	0	0	0	0	-33.8905	150.6988
In situ	NSW1035431	15-295	0	0	4	1	-33.8905	150.6988
In situ	NSW1040890	75-324	0	0	0	0	-33.8906	150.6988
In situ	NSW1035426	14-294	16	8	4	11	-33.8906	150.6989
In situ	NSW1035421	13-293	0	0	0	0	-33.8906	150.6989
In situ	NSW1040901	86-335	0	0	4	1	-33.8906	150.6989
In situ	NSW1040889	74-323	0	0	0	0	-33.8907	150.6988
In situ	NSW1040887	73-322	0	0	0	0	-33.8907	150.6987
In situ	NSW1035416	12-292	0	0	0	0	-33.8907	150.699
In situ	NSW1035294	10-290	0	8	4	5	-33.8907	150.6991
In situ	NSW1040906	87-336	0	0	4	2	-33.8907	150.6989
In situ	NSW1035301	32-312	16	8	4	8	-33.8908	150.6989
In situ	NSW1040902	88-337	0	0	4	2	-33.8908	150.699
In situ	NSW1040728	18-298	0	0	4	3	-33.8903	150.6987
In situ	NSW1035284	9-289	0	0	0	0	-33.8908	150.6991
In situ	NSW1040730	11-291	0	0	0	0	-33.8907	150.699
In situ	NSW1040725	81-330	0	0	0	0	-33.8903	150.6986
In situ	NSW1040903	89-338	0	0	0	0	-33.8908	150.6991
In situ	NSW1040888	72-321	0	0	4	2	-33.8909	150.6987
In situ	NSW1040886	71-320	0	0	0	0	-33.8909	150.6987
In situ	NSW1040885	70-319	0	0	4	2	-33.891	150.6987
In situ	NSW1038832	69-318	0	0	0	0	-33.891	150.6989
In situ	NSW1040884	68-317	0	0	0	0	-33.891	150.6989
In situ	NSW1035285	8-288	0	0	0	0	-33.891	150.6993

	Translocation Options	Tag ID	If pick 16 individuals	If pick 32 individuals	If pick 64 individuals	Unrestricted	Latitude	Longitude
In situ	NSW1035304	29-309	0	8	4	3	-33.8911	150.6989
In situ	NSW1035471	26-306	0	0	4	2	-33.8912	150.6991
In situ	NSW1040883	67-316	0	0	0	0	-33.8912	150.6991
In situ	NSW1035386	27-307	0	0	0	0	-33.8912	150.6991
In situ	NSW1035305	28-308	0	0	4	0	-33.8912	150.699
In situ	NSW1040882	66-315	16	8	4	8	-33.8912	150.6992
In situ	NSW1040909	94-452	0	0	0	0	-33.8913	150.6996
In situ	NSW1035466	25-305	0	0	0	0	-33.8913	150.6994
In situ	NSW1040904	90-339	0	0	0	0	-33.8912	150.6995
In situ	NSW1035461	24-304	0	0	0	0	-33.8914	150.6995
In situ	NSW1040905	91-340	0	0	0	0	-33.8913	150.6995
In situ	NSW1035287	7-A287	16	8	4	16	-33.8914	150.6995
In situ	NSW1040910	95-343	0	8	4	2	-33.8914	150.6995
In situ	NSW1035288	6-286	0	0	4	0	-33.8914	150.6996
In situ	NSW1040908	93-342	0	8	4	5	-33.8914	150.6995
In situ	NSW1040907	92-341	16	8	4	8	-33.8913	150.6995
In situ	NSW1040911	96-344	0	0	0	0	-33.8914	150.6996
In situ	NSW1040912	97-345	0	0	4	0	-33.8914	150.6996
In situ	NSW1040913	98-346	0	0	0	0	-33.8915	150.6996
In situ	NSW1040732	80-A329	0	8	4	5	-33.8902	150.6986
In situ	NSW1035292	4-284	0	0	0	0	-33.8915	150.6996
In situ	NSW1035289	5-285	0	0	0	0	-33.8914	150.6996
In situ	NSW1035293	3-A283	0	0	0	0	-33.8915	150.6996
In situ	NSW1040914	99-347	0	8	4	5	-33.8915	150.6996
In situ	NSW1035357	2-A282	0	0	4	2	-33.8916	150.6996

	Translocation Options	Tag ID	If pick 16 individuals	If pick 32 individuals	If pick 64 individuals	Unrestricted	Latitude	Longitude
In situ	NSW1035290	21-301	0	0	0	0	-33.8916	150.6996
In situ	NSW1035286	22-302	0	0	0	0	-33.8916	150.6996
In situ	NSW1035279	1-A281	0	0	0	0	-33.8916	150.6996
In situ	NSW1040880	64-313	0	0	4	0	-33.8916	150.6995
In situ	NSW1040881	65-314	0	0	4	1	-33.8916	150.6995
In situ	NSW1040875	63-???	0	0	4	0	-33.8916	150.6996
In situ	NSW1040915	100-348	0	8	4	4	-33.8915	150.6996
In situ	NSW1040945	43-K235	0	0	0	0	-33.8944	150.6999
In situ	NSW1040939	38-K230	0	0	0	0	-33.8944	150.7
In situ	NSW1040943	40 K232	0	0	0	0	-33.8944	150.6999
In situ	NSW1040944	42-K234	0	0	0	0	-33.8944	150.6999
In situ	NSW1040935	35-K227	0	0	0	0	-33.8944	150.7001
In situ	NSW1040534	53-K245	0	0	4	0	-33.8945	150.7
In situ	NSW1040951	44-K236	16	8	4	16	-33.8945	150.6999
In situ	NSW1040947	41-K233	0	8	4	6	-33.8944	150.6999
In situ	NSW1040949	48-K240	0	0	0	0	-33.8945	150.6999
In situ	NSW1040946	37-K229	16	8	4	4	-33.8944	150.7
In situ	NSW1040533	52-K244	16	8	4	5	-33.8945	150.7
In situ	NSW1040950	50-K242	0	8	4	6	-33.8945	150.6999
In situ	NSW1040955	51-K243	0	0	4	3	-33.8945	150.7
In situ	NSW1040871	33-K200	0	0	0	1	-33.8944	150.7002
In situ	NSW1040940	39-K231	0	0	4	0	-33.8944	150.6999
In situ	NSW1040942	36-K228	0	0	0	0	-33.8944	150.7001
In situ	NSW1040872	56-K248	0	8	4	4	-33.8945	150.7001
In situ	NSW1040873	57-K249	0	0	0	0	-33.8945	150.7001

	Translocation Options	Tag ID	If pick 16 individuals	If pick 32 individuals	If pick 64 individuals	Unrestricted	Latitude	Longitude
In situ	NSW1040878	61-A279	0	0	0	0	-33.8945	150.7
In situ	NSW1040952	45-K237	0	0	4	0	-33.8945	150.6999
In situ	NSW1040953	47-K239	0	0	4	3	-33.8945	150.6999
In situ	NSW1040874	58-A276	0	8	4	5	-33.8945	150.7001
In situ	NSW1040877	60-A278	16	8	4	13	-33.8945	150.7001
In situ	NSW1040948	46-K238	0	0	4	3	-33.8945	150.6999
In situ	NSW1040536	55-K247	0	0	4	1	-33.8945	150.7
In situ	NSW1040535	54-K246	0	0	0	0	-33.8945	150.7
In situ	NSW1040954	49-K241	16	8	4	5	-33.8945	150.6999
In situ	NSW1040938	34-K226	0	0	4	0	-33.8944	150.7001
In situ	NSW1040876	59-A277	0	0	4	0	-33.8945	150.7001
In situ	NSW1040879	62-A280	0	0	4	0	-33.8946	150.7001
Ex situ	NSW1043179	WSA1 20190019	0	0	0	0		
Ex situ	NSW1043177	WSA2 20190019	0	0	0	0		
Ex situ	NSW1043239	WSA3 20190019	0	0	0	0		
Ex situ	NSW1043187	WSA6 20190019	0	0	0	0		
Ex situ	NSW1043188	WSA7 20190019	0	0	0	0		
Ex situ	NSW1043193	WSA8 20190019	0	0	0	0		
Ex situ	NSW1043183	WSA11 20190019	16	8	4	14		
Ex situ	NSW1043194	WSA12 20190019	0	0	4	0		

	Translocation Options	Tag ID	If pick 16 individuals	If pick 32 individuals	If pick 64 individuals	Unrestricted	Latitude	Longitude
Ex situ	NSW1043186	WSA13 20190019	0	0	0	0		
Ex situ	NSW1043182	WSA14 20190019	0	8	4	4		
Ex situ	NSW1043173	WSA16 20190019	16	8	4	10		
Ex situ	NSW1043175	WSA18 20190019	0	0	4	1		
Ex situ	NSW1043172	WSA19 2018-0773	0	0	0	0		
Ex situ	NSW1043169	WSA21 2018-0773	0	0	4	3		
Ex situ	NSW1043242	WSA28 2018-0793	0	0	4	0		
Ex situ	NSW1043185	WSA9 20190019	0	0	4	0		
Ex situ	NSW1043168	WSA22 2018-0773	0	8	4	3		

Table 5 Translocation example for the tagged individuals at the Western Sydney International Airport site to generate a propagation population size of 100 propagules.

This table lists the original individual to be propagated and the number of cuttings to be obtained from it to reach a total of 100 for an ideal translocated population. The method used follows that for Table 4, which is based on the model presented in Figure 6. Here, an "Unrestricted" starting number of propagated individuals (an ideal case with no limits to the number of starting propagules).

	Translocation Options	Tag ID	Unrestricted	Latitude	Longitude
In situ	NSW1040727	31-A311	1	-33.891	150.6989
In situ	NSW1010741	23-303	1	-33.892	150.6993
In situ	NSW1040729	30-A310	0	33.891	150.6989
In situ	NSW1040736	20-300	4	-33.89	150.6986
In situ	NSW1040897	82-331	0	-33.89	150.6987
In situ	NSW1035441	17-297	0	-33.89	150.6987
In situ	NSW1040892	78-327	0	-33.89	150.6986
In situ	NSW1040731	19-299	0	-33.89	150.6986
In situ	NSW1040894	79-328	2	-33.89	150.6986
In situ	NSW1035436	16-296	4	-33.89	150.6988
In situ	NSW1040891	77-326	2	-33.89	150.6987
In situ	NSW1040898	83-332	2	-33.89	150.6988
In situ	NSW1040899	84-333	0	-33.89	150.6988
In situ	NSW1040900	85-334	4	-33.891	150.6988
In situ	NSW1040893	76-325	0	-33.891	150.6988
In situ	NSW1035431	15-295	0	-33.891	150.6988
In situ	NSW1040890	75-324	0	-33.891	150.6988
In situ	NSW1035426	14-294	4	-33.891	150.6989
In situ	NSW1035421	13-293	0	-33.891	150.6989
In situ	NSW1040901	86-335	1	-33.891	150.6989
In situ	NSW1040889	74-323	0	-33.891	150.6988
In situ	NSW1040887	73-322	0	-33.891	150.6987
In situ	NSW1035416	12-292	0	-33.891	150.699
In situ	NSW1035294	10-290	2	-33.891	150.6991
In situ	NSW1040906	87-336	1	-33.891	150.6989
In situ	NSW1035301	32-312	3	-33.891	150.6989
In situ	NSW1040902	88-337	1	-33.891	150.699
In situ	NSW1040728	18-298	1	-33.89	150.6987
In situ	NSW1035284	9-289	0	-33.891	150.6991
In situ	NSW1040730	11-291	0	-33.891	150.699
In situ	NSW1040725	81-330	0	-33.89	150.6986

	Translocation Options	Tag ID	Unrestricted	Latitude	Longitude
In situ	NSW1040903	89-338	0	-33.891	150.6991
In situ	NSW1040888	72-321	1	-33.891	150.6987
In situ	NSW1040886	71-320	0	-33.891	150.6987
In situ	NSW1040885	70-319	1	-33.891	150.6987
In situ	NSW1038832	69-318	0	-33.891	150.6989
In situ	NSW1040884	68-317	0	-33.891	150.6989
In situ	NSW1035285	8-288	0	-33.891	150.6993
In situ	NSW1035304	29-309	2	-33.891	150.6989
In situ	NSW1035471	26-306	1	-33.891	150.6991
In situ	NSW1040883	67-316	0	-33.891	150.6991
In situ	NSW1035386	27-307	0	-33.891	150.6991
In situ	NSW1035305	28-308	0	-33.891	150.699
In situ	NSW1040882	66-315	3	-33.891	150.6992
In situ	NSW1040909	94-452	0	-33.891	150.6996
In situ	NSW1035466	25-305	0	-33.891	150.6994
In situ	NSW1040904	90-339	0	-33.891	150.6995
In situ	NSW1035461	24-304	0	-33.891	150.6995
In situ	NSW1040905	91-340	0	-33.891	150.6995
In situ	NSW1035287	7-A287	4	-33.891	150.6995
In situ	NSW1040910	95-343	1	-33.891	150.6995
In situ	NSW1035288	6-286	0	-33.891	150.6996
In situ	NSW1040908	93-342	2	-33.891	150.6995
In situ	NSW1040907	92-341	3	-33.891	150.6995
In situ	NSW1040911	96-344	0	-33.891	150.6996
In situ	NSW1040912	97-345	0	-33.891	150.6996
In situ	NSW1040913	98-346	0	-33.892	150.6996
In situ	NSW1040732	80-A329	2	-33.89	150.6986
In situ	NSW1035292	4-284	0	-33.892	150.6996
In situ	NSW1035289	5-285	0	-33.891	150.6996
In situ	NSW1035293	3-A283	0	-33.892	150.6996
In situ	NSW1040914	99-347	3	-33.892	150.6996
In situ	NSW1035357	2-A282	1	-33.892	150.6996
In situ	NSW1035290	21-301	0	-33.892	150.6996
In situ	NSW1035286	22-302	0	-33.892	150.6996
In situ	NSW1035279	1-A281	0	-33.892	150.6996
In situ	NSW1040880	64-313	0	-33.892	150.6995
In situ	NSW1040881	65-314	0	-33.892	150.6995
In situ	NSW1040875	63-???	0	-33.892	150.6996
In situ	NSW1040915	100-348	2	-33.892	150.6996

	Translocation Options	Tag ID	Unrestricted	Latitude	Longitude
In situ	NSW1040945	43-K235	0	-33.894	150.6999
In situ	NSW1040939	38-K230	0	-33.894	150.7
In situ	NSW1040943	40 K232	0	-33.894	150.6999
In situ	NSW1040944	42-K234	0	-33.894	150.6999
In situ	NSW1040935	35-K227	0	-33.894	150.7001
In situ	NSW1040534	53-K245	0	-33.895	150.7
In situ	NSW1040951	44-K236	4	-33.895	150.6999
In situ	NSW1040947	41-K233	3	-33.894	150.6999
In situ	NSW1040949	48-K240	0	-33.895	150.6999
In situ	NSW1040946	37-K229	2	-33.894	150.7
In situ	NSW1040533	52-K244	2	-33.895	150.7
In situ	NSW1040950	50-K242	3	-33.895	150.6999
In situ	NSW1040955	51-K243	2	-33.895	150.7
In situ	NSW1040871	33-K200	0	-33.894	150.7002
In situ	NSW1040940	39-K231	0	-33.894	150.6999
In situ	NSW1040942	36-K228	0	-33.894	150.7001
In situ	NSW1040872	56-K248	2	-33.895	150.7001
In situ	NSW1040873	57-K249	0	-33.895	150.7001
In situ	NSW1040878	61-A279	0	-33.895	150.7
In situ	NSW1040952	45-K237	0	-33.895	150.6999
In situ	NSW1040953	47-K239	1	-33.895	150.6999
In situ	NSW1040874	58-A276	2	-33.895	150.7001
In situ	NSW1040877	60-A278	4	-33.895	150.7001
In situ	NSW1040948	46-K238	1	-33.895	150.6999
In situ	NSW1040536	55-K247	0	-33.895	150.7
In situ	NSW1040535	54-K246	0	-33.895	150.7
In situ	NSW1040954	49-K241	3	-33.895	150.6999
In situ	NSW1040938	34-K226	0	-33.894	150.7001
In situ	NSW1040876	59-A277	0	-33.895	150.7001
In situ	NSW1040879	62-A280	0	-33.895	150.7001
Ex situ	NSW1043179	WSA1 20190019	0		
Ex situ	NSW1043177	WSA2 20190019	0		
Ex situ	NSW1043239	WSA3 20190019	0		
Ex situ	NSW1043187	WSA6 20190019	0		
Ex situ	NSW1043188	WSA7 20190019	0		
Ex situ	NSW1043193	WSA8 20190019	0		
Ex situ	NSW1043183	WSA11 20190019	4		
Ex situ	NSW1043194	WSA12 20190019	0		

	Translocation Options	Tag ID	Unrestricted	Latitude	Longitude
Ex situ	NSW1043186	WSA13 20190019	0		
Ex situ	NSW1043182	WSA14 20190019	2		
Ex situ	NSW1043173	WSA16 20190019	4		
Ex situ	NSW1043175	WSA18 20190019	0		
Ex situ	NSW1043172	WSA19 2018-0773	0		
Ex situ	NSW1043169	WSA21 2018-0773	1		
Ex situ	NSW1043242	WSA28 2018-0793	0		
Ex situ	NSW1043185	WSA9 20190019	0		
Ex situ	NSW1043168	WSA22 2018-0773	1		

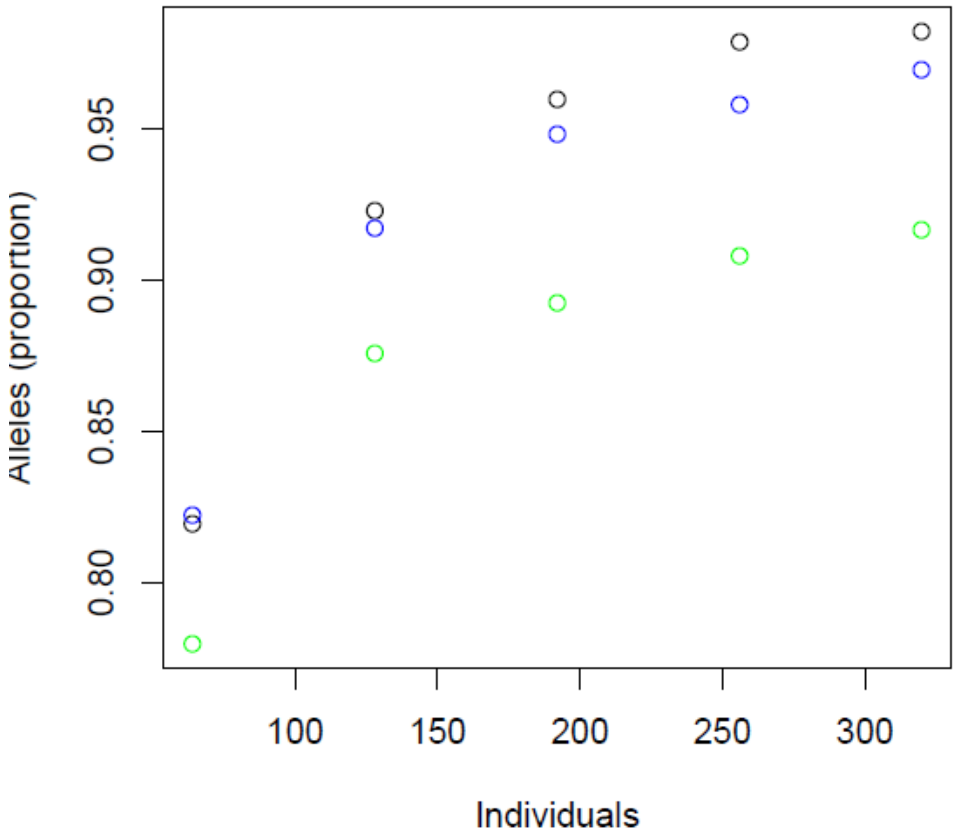


Figure 8: Simulations of genetic polymorphism for translocation populations of different sizes at the Western Sydney International Airport (WSI) site of *Pimelea spicata*.

Potential translocation populations of different sizes (64, 128, 192, 256, 320 individuals) were assembled using propagation populations of different sizes (16, 32 and 64 individuals). For example, to construct a translocation population of 256 individuals from a propagation population of 32 individuals, 8 ramets (256/32=8) of each individual in the propagation population are used. Each translocation population was simulated forward for 10 generations. At the conclusion of the simulation, the number of SNP loci that remained polymorphic was calculated, and expressed as a proportion of the total number of loci where the minor allele was common (> 2%) at the airport. These proportions are plotted as a function of the size of the translocation population, for propagation populations of 16 (green), 32 (blue) and 64 (black) individuals (respectively).

This plot suggests that in a translocation population of more than 256 propagules, a substantial proportion of the initial diversity will remain after 10 generations. We therefore adopt 256 individuals as the recommended size of the translocation population (Table 4). When a translocation population of this size was generated from 32 genetically selected individuals, greater than 95% of variation was maintained.

Given the large number of individuals at the WSI site, we also explored a translocation option involving 256 plants to be selected from the airport individuals (Table 4). The number of ramets per genet was restricted to having a maximum of 16 so that translocation does not depend on the mass propagation of any given genet.

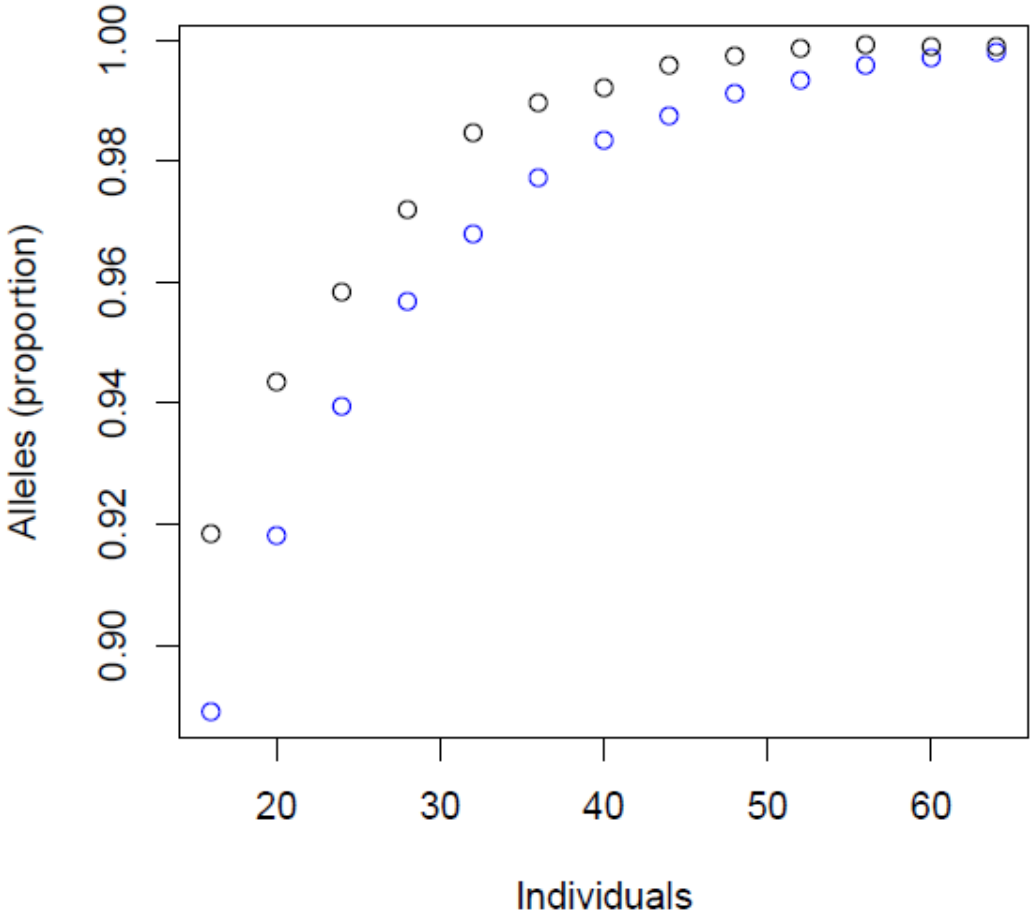


Figure 9: The proportion of loci that are polymorphic in populations of different sizes at the Western Sydney International Airport (WSI) site of *Pimelea spicata*.

Different numbers of propagules were selected from WSI by optimising on the basis of genetic diversity (black symbols) and by choosing at random (blue symbols, representing means of 20 replicates).

This shows that a genetic-based approach will enable us to maximise diversity from a smaller number of individuals (i.e. we can select just over 30 individuals to maintain over 95% of the population diversity). On average, the optimised populations conserve more allelic diversity than randomly selected sets of individuals, but this effect is greatest when the number of genets is relatively small.

That is, if 64 individuals are included in the population, most common alleles at WSI tend to be captured. Conversely, if 16 individuals are included, the choice of those individuals makes a larger difference to the proportion of common alleles represented in the population.

6. REFERENCES

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