Conserving wild population diversity in the Desert Quandong of central Australia

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Santalum acuminatum at Mulga Park, Petermann, Northern Territory. Image by Caroline Chong.

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Summary

This project contributes to the conservation of the Desert Quandong, *Santalum acuminatum* (Santalaceae), by improving knowledge of the genetic diversity of wild populations in central Australia. This iconic desert species is highly valued by Aboriginal peoples and it is now under commercial production for the bush tucker industry. Relatedly, the species is increasingly recognised for its tourism value. Overall, people have a close affinity with quandong, and a deep interest in it.

The Desert Quandong is impacted by multiple threats across its range. In central Australia, many quandong stands are in poor health due to repeated fire, to browsing and trampling by camels, cattle and rabbits, and to past over-harvesting in some areas. Buffel Grass also negatively impacts this species at some sites, by increasing fire severity and frequency, reducing host plant abundance and crowding out young plants. Long term outcomes for the future of Desert Quandong include management actions maintaining and encouraging wild populations building resilience. In addition, population augmentation and translocation are identified recovery actions and Aboriginal peoples wish to see this bush tucker species thriving again on country. Genetic information produced from this project will inform decision making leading to these outcomes including the selection of appropriate source populations for species recovery actions in the Northern Territory. This information will additionally help to clarify the national conservation status of Desert Quandong populations, a listed Vulnerable threatened species in the Northern Territory.

Through this project, we applied a panel of highly informative genetic markers developed specifically for the Desert Quandong to identify population genetic diversity and population structure. This project also establishes a significant molecular data resource for future research and monitoring of the Desert Quandong. A key finding from this study is that the Desert Quandong in the Northern Territory represents two main genetic lineages. These two lineages are geographically based, represented by distinct stands at Mulga Park and Watarrka National Park. Notably, a pattern of admixture at other sites sampled in the NT indicates past gene flow between the extant geographically separated stands and large extents of occurrence of the two lineages. Further, our findings show a generalised pattern of directional relative migration from the Northern Territory southwards into South Australia. This directional movement of genes is consistent with the scenario that the Desert Quandong in the northern extent of its range comprises a series of naturally patchy, remnant populations that contribute significantly to the persistence of the species.

Priorities for conservation management of this important species in the NT include integrated on-ground management efforts to protect significant remnant stands, including the genetic lineages represented at Mulga Park and Watarrka National Park. Beyond this, in informing recovery actions for the species, Mulga Park is identified as a genetically appropriate source location of material for augmentation of the Desert Quandong on Uluru Kata-Tjuta Park. Future planning for recovery actions aiming to bolster the availability of Desert Quandong through orcharding or to protect the wild genetic diversity of the species should prioritise inclusion of both lineages, including Watarrka National Park to ensure adequate representativeness. Further genetic research and assessment of remnant stands in the NT and northern SA will help to clarify the relative diversity of genetic lineages of the Desert Quandong in central Australia. Doing so can inform decisions on recovery actions to support the persistence of wild populations into the future.

Introduction

A member of the sandalwood family Santalaceae, the Desert Quandong occurs in semi-arid Australia. In the NT, it occurs as a series of discrete subpopulations, mainly in the south-west. One of the largest occurrences is at Watarrka National Park, while other significant stands occur on pastoral properties and on Aboriginal lands. The population at Uluru- Kata Tjuta National Park is small (eight plants), but of high cultural significance.

The species is listed as Vulnerable in the Northern Territory under the Territory Parks and Wildlife Conservation Act 1976. Past over-harvesting for woodcarving, browsing impacts on adults and seedlings and fire are long recognised threats to this species in central Australia (NT and SA). More recently, however, climate change impacts have become apparent. Specifically, Park Rangers reported significant drought mortality in the Watarrka National Park population over the 2019-2020 period. This coincided with one of the driest and hottest period in recent history; an outcome that is consistent with climate change predictions for central Australia. Changes in rainfall seasonality is a potential further threat to this species. Reduced winter rainfall could result in lower seedling recruitment given that this species requires germination under cooler temperatures. Climate change represents a significant present and future threat to this species, and coupled with other threats, could lead to a significant range contraction.

Importantly, Desert Quandong is not currently listed under Commonwealth EPBC legislation. Clarification of the species' national conservation status is needed to implement appropriate conservation actions and management planning. In central Australia, population decline, evidenced by observation as well as poor recruitment and demographic monitoring (Nano et al. 2020), is projected to escalate under current threats and climate-driven range contraction. There is limited knowledge of the genetic diversity within the NT population and it is unclear if the NT population is genetically discrete from neighbouring state populations. In other populations of *Santalum acuminatum* across its entire range, recruitment often appears limited and the research results developed in this study may be applicable to other states and regions.

The project investigators have recently undertaken a successful pilot study incorporating NT specimens from three locations to develop a panel of highly informative genetic markers for the Desert Quandong. The targeted genotying approach has demonstrated efficacy for population differentiation in the species and within-population genetic structure assessments. Applying these molecular tools, this project will contribute new robust information on population diversity as well as establish a significant molecular data resource for future research and monitoring of the Desert Quandong. The highest priority now is to investigate genetic relationships within the largest known NT population at Watarrka National Park. Potentially, propagules from this population can be used to bolster declining stands at other locations, but currently, data are not available to assess the risks and benefits of this approach.

A strong network of partner organisations is already committed to improving the long-term outlook for wild stands of this species in the NT. Critically, though, plans to implement conservation actions relating to population re-introduction and augmentation are stalled due to inadequate knowledge of genetic relationships among stands. Improved understanding will directly inform the choice of source populations for propagule production.

Project aims and significance

This project aims to assess genetic diversity, and determine population distinctiveness of the *Santalum acuminatum* (Quandong) population at Watarrka National Park. The information derived will inform population translocation and augmentation planning at Watarrka and potentially at other locations and the ongoing management of the species.

At Watarrka National Park, the Quandong population occurs on a high rugged sandstone plateau and in the surrounding sand country in discrete catchments. Both occurrences were severely damaged by short-interval fire over the 2011-2013 period, though the sand country stands were worst affected, with high levels of mortality and slow rates of recovery. Severe drought and above average summer temperatures during 2019-2020 further contributed to the poor condition of the entire population. This is a poor conservation and cultural outcome given that Aboriginal people now have limited opportunities to harvest this important species from accessible areas. As a result, there is a strong desire to establish plantings in nearby communities and to augment the diminished stands on Park. Information on stand relatedness is therefore needed to guide this process such that potential adverse genetic outcomes can be avoided.

This project's relevance extends beyond the immediate issue of the best practice management of the Watarrka population. Similar planning is underway elsewhere in the NT and there is therefore some urgency to resolve questions relating to appropriate source populations for translocation.

Knowledge of how genetic variation is distributed geographically will help managers to identify priority seed sources for other translocations and where threat mitigation and propagation efforts need to be focused to conserve priority wild populations in the NT. The research findings from this project will guide on-ground efforts to augment wild populations and support long-term population viability. In fact, knowledge of this iconic Australian bush food species will be of value to the ongoing needs to maintain and manage its presence in the Australian bush.

Research methodology

General approach

This project aimed to 1) assess the genetic variability of the Watarrka population of Desert Quandong (*Santalum acuminatum*) using samples from two catchments 2) determine the genetic relatedness of the Watarrka population to other NT populations 3) place this genetic variability and relatedness in the context of facilitating conservation and management of this species' remaining populations in the NT.

The project used samples collected through a network of project collaborators and supportive industry and traditional owner groups from wild populations and applied highly informative genomic-based genotyping methods to build a shared knowledge base of wild population genetic diversity in *Santalum acuminatum*.

Study design

Population samples were sourced through existing and new samples collected under investigator collaborative partnerships. Samples from the focus population, Watarrka National Park, (currently available 32 individuals), and regional reference samples collated and

analysed from nine locations (106 samples) in the NT and northern SA were included in this study.

High-quality Northern Territory Herbarium and South Australia State Herbarium databased specimens of *Santalum acuminatum* representing a wide geographical range (54 samples) were included and used to contextualise the allelic diversity detected in the focal NT stands. For example, plants identified as being from locations where Quandong is currently absent may be used to ascertain loss of genetic diversity due to population loss over time. This will inform the scale of connectivity evident in this species, which is expected to be moderate to high due to the potential for long-distance dispersal by birds and some other animals that eat the fruits of Quandong.

The geographical distance between sampling sites is more than 50 km and the minimum distance between individual samples within sites is 5 m from a single stem/trunk, to optimise capture of different genetic individuals while accounting for typical stand structure where there are multi-stemmed stands.

Study location and methods

Leaf tissue samples were obtained from *Santalum acuminatum* stands from individual stems from Watarrka National Park, an additional two pastoral and one national park locations in the NT. These four locations, Mulga Park, Curtin Springs, Uluru-Kata Tjuta National Park and Watarrka National Park were separated by distances of between 60 – 140 km. Individual stems were sampled from a minimum of 5 m apart to optimise capture of different genetic individuals. A minimum of 30 individual samples per location were targeted for collection to enable adequate representation of potential allelic variation, dependent on the total number of individuals at each location. Thirty-two individual samples were collected from Watarrka National Park from two catchments separated by 4 km, Kathleen Creek and Stokes Creek (Figure 1). The GPS location of each sample was recorded along with demographic data (height, stem circumference, reproductive status, evidence for past reproduction, evidence for disturbance). Leaf tissue was sampled immediately into labelled teabags and placed into ziplock bags containing silica gel beads to facilitate rapid drying and DNA preservation prior to transport to the laboratory.

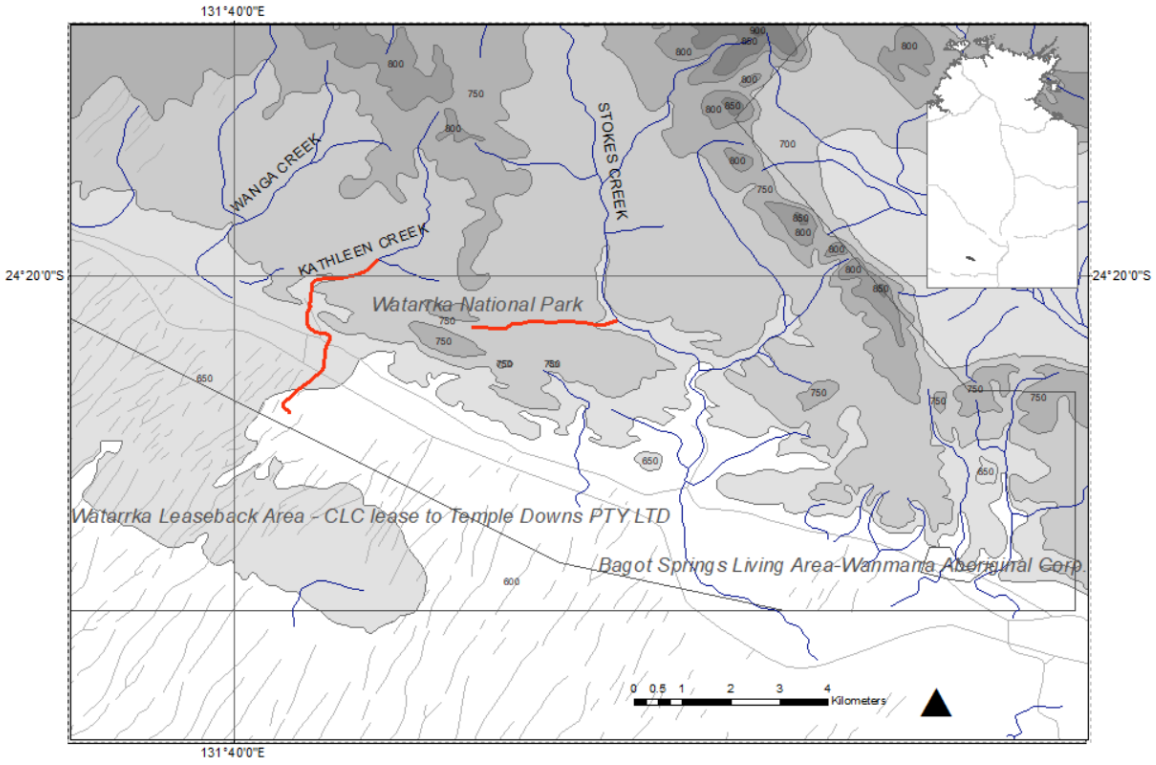

Figure 1. Map of collection area of *Santalum acuminatum* leaf samples for genetic analysis at Watarrka National Park in the Northern Territory, Australia. Samples were collected from two catchments (red lines), Kathleen Creek and Stokes Creek. Insert at top right shows the location of Watarrka National Park in the south-west of the Northern Territory. Darkest grey shading is highest elevation on the George Gill Range, lightest shading is lowest elevation on the surrounding sand dune land system (fine lines are sand ridges).

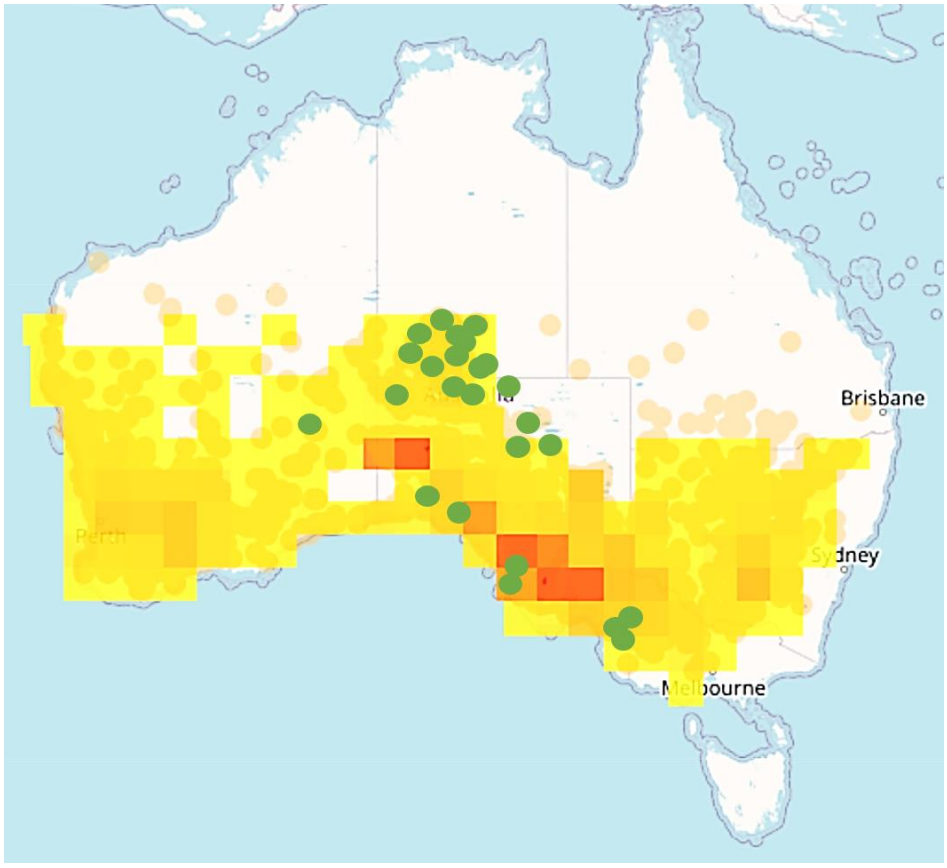

Figure 2. Map of collection sites and herbarium record locations of *Santalum acuminatum* leaf samples for genetic analysis from sites in the NT, SA and WA. Green filled circles represent sampled sites; pale orange filled circles represent all recorded observations of the species; density map represents relative density of the records. Map created using data from Atlas of Living Australia (website accessed December 2023, https://www.ala.org.au, ©OpenStreetMap contributors).

Figure 3a, 3b. Heavily browsed Desert Quandong and undertaking of demographic assessment at Mulga Park, Northern Territory. Images by Caroline Chong.

Data

Molecular methods

Appropriate next-generation genomic-generated molecular techniques and protocols applied in this study were developed for *Santalum acuminatum* through University of Adelaide student research training (Mr Alex Stuart, Semester 2, 2021) under the direction of Kor-jent van Dijk and Michelle Waycott supported by Caroline Chong and Catherine Nano. This preliminary work was undertaken to assess the feasibility of the proposed approach and provide applied research experience for a talented undergraduate student. The technical approach required the application of the ddRADseq (double digest Restriction Associated DNA sequencing) protocol (Peterson *et al*. 2012). This protocol was used to generate polymorphic species-specific gene regions that were used to develop primers for the subsequent genotyping protocol. We applied the MassARRAY® (Ellis and Ong 2017) approach and screening was undertaken in the University of Adelaide and Australian Genome Research Facility using a paired-end Illumina sequencing protocol. SNP selection for MassARRAY® primer design was undertaken using the Qiagen CLC Workbench. Samples are screened through services provided by AGRF (Australian Genome Research Facility). The MassARRAY® system for SNP (single nucleotide polymorphism) genotyping was conducted on all samples, using markers previously identified and tested. MassARRAY® uses mass spectroscopy, a technique that measures the mass of molecules within a sample; in this case short DNA fragments containing known SNP sites. The samples were screened across a bank of 50 tested, identified polymorphic SNP loci. Pilot study data (Alex Stuart, 2020) for samples including NT locations indicates average allele frequencies being between 1.3-1.9 alleles per locus (noting for this method that two alleles per locus is typically the maximum). This pilot study also identified the potential for these markers to resolve clonal identity although that it outside the scope of this proposed study.

Data management and communication

Biological specimen data

All leaf tissue samples are stored under appropriate laboratory conditions at the Northern Territory Herbarium and the State Herbarium of South Australia. Following conventional protocol for flora specimen data management, all sample collection data from NT locations were databased electronically on the NT Vegetation Site Database and will be made publicly accessible via the online GIS portal NRMaps. All sample collection data from SA locations will be databased and included in the State Herbarium database delivered to the AVH (avh.chah.org.au). Molecular sequence data and associated metadata will be made publicly available via sequence read archives.

Molecular data analysis

MassARRAY® SNP genotyping

Genotyping was performed by the MassARRAY system using 50 SNP markers distributed across the genome in 176 individuals of *Santalum acuminatum*. Of the 50 markers that were used in the MassARRAY, 43 produced successful results.

Detection of Clonality

The raw SNP calls were converted into the correct format and imported into Genodive 3.04. GenoDive was subsequently used to identify clonality within these nine populations, with all 176 individuals and 46 loci used in this analysis. The clonality detection test conducted by

this software determines the probability of finding the observed clonal diversity under random mating. The algorithm used in this test first calculates a matrix of genetic differences, and chooses a threshold distance; if the distance between a pair of individuals is below this threshold, they are designated as clonal, and all clonal individuals are referred to as the same genet (genetic individual). The test statistic in this case is the Corrected Nei's Diversity Index, which corresponds to the average diversity per locus, *Hs*. The Clonal Diversity Index (*R*) is equal to the ratio of number of genets to the number of sampled individuals, $R = (C-1)/(N-1)$. The alleles for all individuals were randomised during this test. Overall, the number of unique individuals observed using a threshold of 1 was 93.

Allele Frequencies
In all 43 loci sampled, only two possible bases were detected for each allele, leading to 3 possible genotypes at each locus. While there were no alleles that were only found in a single population, the rate of occurrence of some alleles was rare relative to all individuals: 9/43 loci returned reads that showed a 19:1 or lower base ratio for all reads.

Measures of Genetic Diversity
Having removed individuals identified as clones, population genetic indices for populations were calculated using GenoDive, including Observed Heterozygosity, Expected Heterozygosity and an Inbreeding Coefficient. This was done on a population basis. The Observed Heterozygosity (Ho) is the frequency at which heterozygotes appear in a population, where 0 contains only homozygotes, and 1 contains only heterozygotes. The Heterozygosity Within Populations (Hs) refers to the expected frequency of heterozygotes if that population is at Hardy-Weinberg equilibrium. The Inbreeding Coefficient, Gis, gives the probability that any two alleles at a locus of an individual are identical by descent (Gis = (HS - HO)/Hs), and can be used to quantify genetic diversity.

Population Differentiation
Estimates of population differentiation in GenoDive were based on pairwise Fst tests, between different populations of *S. acuminatum*. Fst corresponds to the average number of pair-wise differences in alleles between individuals. Lower Fst values indicate two populations display less allelic variance and are thus more closely related.

Discriminant analysis of principal components

Discriminant analysis of principal components (DAPC) implemented in the R package adegenet (Jombart 2008) was used to visualise population genetic structuring. An initial DAPC was run considering 100 principal components. The optim.a.score() method was subsequently used to assess the optimal number of PCs to retain and a second DAPC analysis was conducted. The proportion of total variance explained by the first two principal components was also calculated.

Population structure

We employed STRUCTURE version 2.3.4 (Pritchard et al. 2000) to examine the assignment of individuals to distinct genetic clusters and infer the extent of admixture among geographically defined populations. We applied a hierarchical approach to the analyses. In summary, we firstly examined the assignment of all 176 individuals in the data set to genetic clusters with K varying from 1 to 14. We subsequently ran STRUCTURE using subsets of the data to examine evidence for fine-scale genetic structure, representing 1) only those samples from the NT, with 83 individuals and K varying from 1 to 6; 2) only those samples from NT and SA with greater than two individuals per site from 11 geographically defined populations, with

136 individuals and K varying from 1 to 11. All STRUCTURE runs used 10 replications with a burnin of 500,000 followed by 1,000,000 MCMC iterations. The most plausible values of K were determined using the delta K statistic in CLUMPAK (Evanno et al. 2005; Kopelman et al. 2015) and visualisation of the degree of admixture. All STRUCTURE analyses were conducted using the University of Adelaide Phoenix High Performance Computing infrastructure.

Relative migration and directional gene flow

To investigate evidence for significant directional migration between geographic populations, we used relative migration analysis in the R package diveRsity (Keenan et al. 2013). Relative migration between population pairs was estimated with the divMigrate function using the differentiation statistics d (Jost's D), gst (Nei's Gst) and Nm (number of migrants; Alcala et al. 2014) applying the method of Sundqvist et al. 2013. The value and significance of pairwise relative migration was tested using bootstrap iterations and calculation on non-overlapping confidence intervals (95% CIs). A weighted network was plotted representing the relative migration levels between all pairs of populations. The geographic populations analysed were a subset of the data, being those where the minimum number of individuals per population was ten.

Results

Genetic diversity

Table 1. Results of diversity analysis using GenoDive of genetic individuals of *Santalum acuminatum* sampled from the NT, SA and WA. The statistics are population estimates of N_alleles: average number of alleles; N_effective: effective number of alleles; Ho: observed heterozygosity; Hs: expected heterozygosity at Hardy-Weinberg equilibrium; G_{IS}: inbreeding coefficient (Hs – Ho)/Hs.

Population	N_alleles	N_effective	Ho	Hs	G_{IS}
UKTNP	1.53	1.24	0.156977	0.165504	0.051522
Kathleen_North	1.33	1.25	0.197674	0.166667	-0.186047
Kathleen_South	1.395349	1.212375	0.158869	0.135013	-0.176692
Upper Stokes	1.418605	1.196674	0.142085	0.123228	-0.153031
Mulga Park	1.534884	1.288539	0.165232	0.175584	0.058961
Curtin Springs	1.558140	1.298945	0.195549	0.191859	-0.019231
Brookfield	1.627907	1.347718	0.291860	0.224419	-0.300518
Murray	1.744186	1.490601	0.340310	0.310465	-0.096130
Murray_501	1.452381	1.312454	0.285714	0.206349	-0.384615
Nullabor	1.697674	1.325565	0.199548	0.213557	0.065598
Nullabor	1.790698	1.406072	0.219404	0.250775	0.125094
Eyre1	1.365854	1.246529	0.142276	0.191057	0.255319
Eyre2	1.697674	1.406936	0.255094	0.260437	0.020517
Eyre3	1.860465	1.501880	0.297457	0.309446	0.038742
WA	1.325581	1.177332	0.117054	0.117442	0.003300

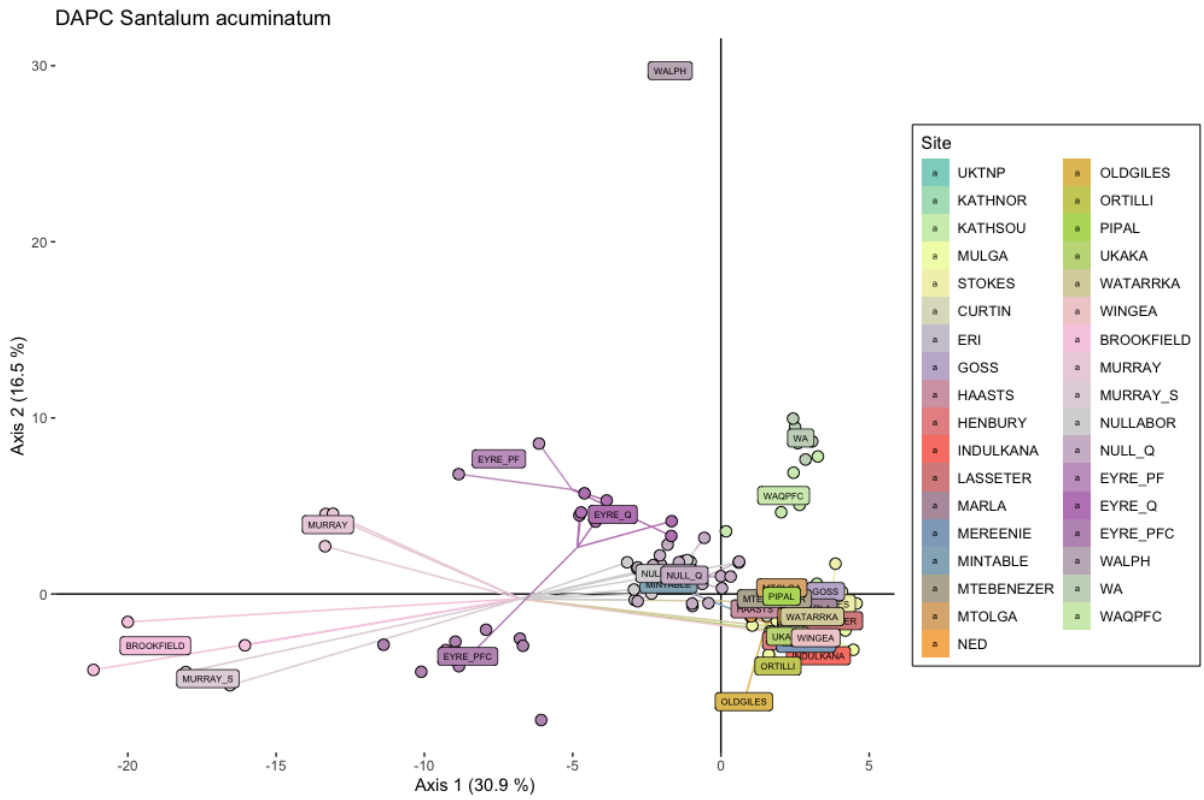

Figure 4. Discriminant analysis of principal components (DAPC) plot depicting variation among 176 individuals of *Santalum acuminatum* genotyped across 43 MassARRAY SNPs.

Population structure

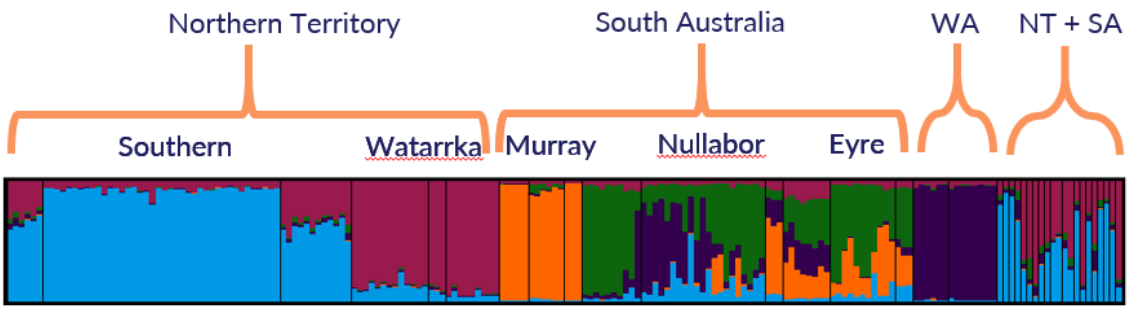

Figure 5a. Results of population structure analyses from STRUCTURE (Pritchard et al. 2000). Genetic clusters for all individuals sampled from the NT, SA, and WA; vertical columns represent a samped individual, colour-coded for assigned cluster at K = 8.

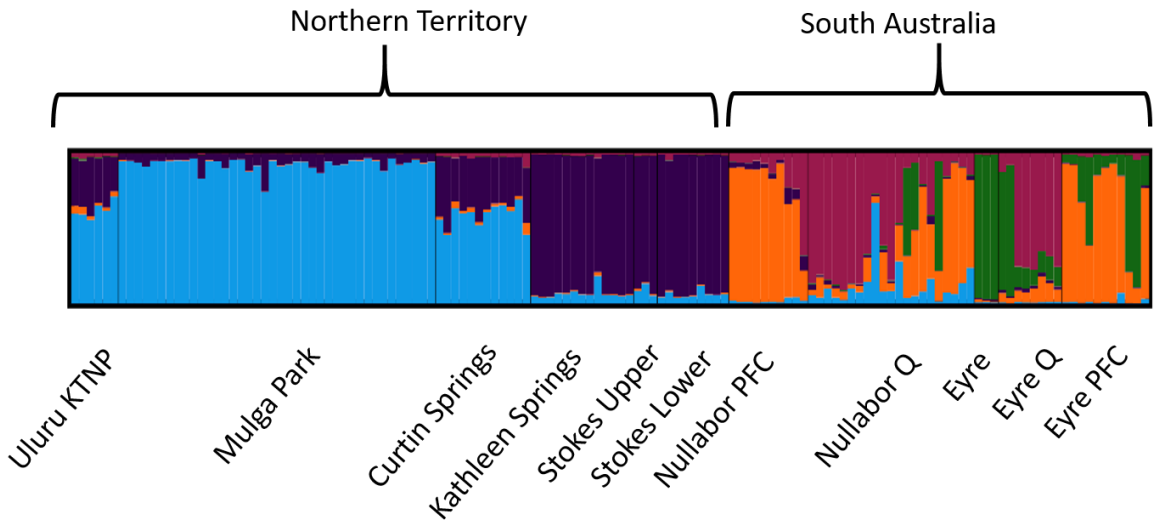

Figure 5b. Results of hierarchical population structure analyses from STRUCTURE (Pritchard et al. 2000). Genetic clusters for all geographic populations sampled from the NT and SA, excluding three highly differentiated populations from the River Murray district; vertical columns represent a samped individual, colour-coded for assigned cluster at K = 5.

Relative migration

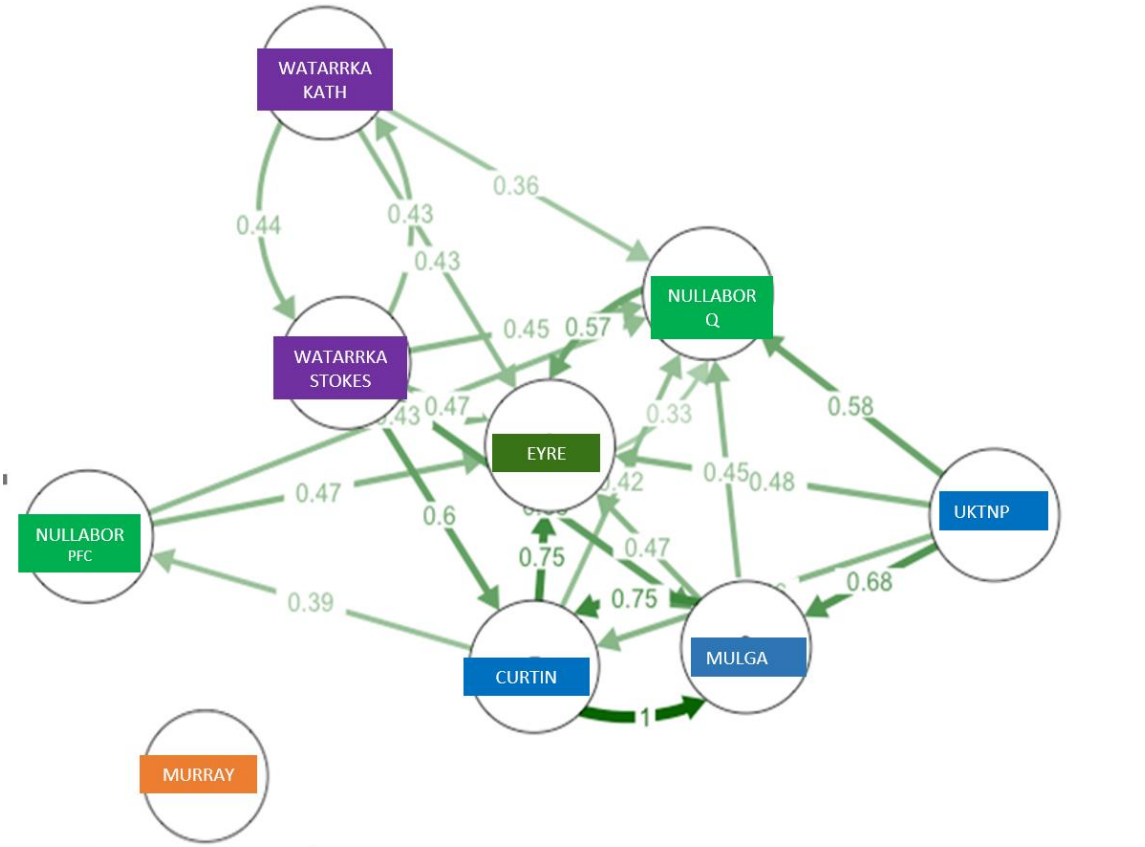

Figure 6. Results of relative migration analysis from diveRsity (Keenan et al. 2013). The weighted network plot represents the relative migration levels between all pairs of populations, estimated using the number of migrants Nm (Alcala et al. 2014). Labelled circles represent geographic populations analysed, being those where the minimum number of individuals per population was ten. Values and arrows represent significant directional migration between population pairs, with only those values greater than 0.3 displayed for clarity.

Genetic diversity and differentiation

Targeted SNP genotyping using MassARRAY® was applied in this study as an appropriate method to detect population structure and differentiation. Our hierarchical sampling design enabled assessment of the relative partitioning of genetic diversity across geographically based sites in the NT, SA and WA.

Overall, the sampled stands showed comparable levels of population diversity and no significant levels of inbreeding as estimated using the metrics of observed and expected heterozygosity and inbreeding coefficient (Gis). From the Northern Territory, Kathleen Creek and Stokes Creek at Watarrka National Park and Curtin Springs had similar Gis values of < 0.0, indicating a slight excess of heterozygotes and

very low rates of inbreeding, while Uluru Kata-Tjuta and Mulga Park had Gis values of < 0.1, indicating low rates of inbreeding. Stands from WA and the Eyre Peninsula and Nullabor in South Australia showed positive but relatively low values of inbreeding (Table 1).

Stands of Desert Quandong were determined to be differentiated at the regional scale. Evidence for this differentiation was high values for pairwise Fst estimation of genetic differentiation as well as the genetically differentiated clusters recovered using discriminant analysis of principal components (Figure 4). The greatest Fst values indicating strongest genetic differentiation were between populations from the River Murray district in south-eastern South Australia to stands from Watarrka National Park in the NT, with all pairwise Fst values > 0.40. Within the NT, pairwise Fst values between stands were relatively low and ranged between 0.02 – 0.08, with Curtin Springs and Uluru Kata-Tjuta showing no population differentiation (pairwise Fst values < 0.0). The two sampled stands from eastern WA also showed strong differentiation to the other regions, in particular the River Murray district and Northern Territory (pairwise Fst values 0.3 – 0.6). The pattern of regional genetic differentiation was also evident through DAPC visualisation (Figure 4). The higher degree of clustering of stands from the NT relative to those from SA (Figure 4) may be partially explained by the different geographic extent represented in the SA and NT samples.

Genetic structure

The estimation of individual assignment to distinct genetic clusters using STRUCTURE revealed a hierarchical population structure. At the regional scale, individuals from the NT, southern SA and WA were grouped to distinct genetic clusters (average admixture < 0.30; Figure 5a). The estimation supported an overall best K-value of 5 or 8. Two genetic lineages were detected from the NT, with Mulga Park and Watarrka National Park being the most distinct whereas two other sites from the NT, Uluru-Kata-Tjuta and Curtin Springs, showed a level of admixture between the two lineages (Figure 5b). The extent of population distinctiveness was lesser among most of the SA sites sampled, with the exception of the River Murray district. Importantly, we also included herbarium specimens representing individuals and pairs of samples from a wide range of available locations from NT and northern SA to increase the geographic representativeness of our sample set (Figure 5a, right-most panel). While not sampled at the population scale, inclusion of these samples showed that considerable genetic mixing across NT and northern SA sites may have occurred in the past.

Directional gene flow

Investigation of significant relative migration between geographically defined population pairs revealed significant directional migration in the sampled network of sites (Figure 6). The pattern of asymmetric gene flow shows 1) the significantly directional movement of genes from NT sites to SA sites when considered using the statistic of number of migrants; 2) high levels of shared migrants between Uluru Kata-

Tjuta, Curtin Springs and Mulga Park in the NT; 3) high levels of shared migrants between Kathleen Creek and Stokes Creek in Watarrka National Park in the NT; 4) lowest levels of shared migrants between the River Murray and all other analysed sites.

Discussion

In this study, distinct genetic lineages of the Desert Quandong were detected from the Northern Territory, Western Australia and from the River Murray district of South Australia. The two main genetic lineages detected from the Northern Territory are geographically based, and are represented by stands from Mulga Park and from two stream lines from Watarrka National Park. These stands are among the largest remnant stands known from the NT, with approximately 45 mature individuals at Mulga Park and more than 100 individuals at Watarrka National Park. The stands at Uluru Kata-Tjuta National Park and at Curtin Springs are more admixed between the two lineages, indicating past gene flow among sites. Notably, a pattern of admixture at other sites sampled in the NT at the individual scale (herbarium specimens) also indicates past gene flow between geographically separated stands. The pattern of admixture supports the expectation that each of the two lineages have geographic representation beyond the extent of sites sampled in this study.

The evidence for admixture across geographically distant areas and remnant stands in the Northern Territory is consistent with previous research on the diversity of *Santalum acuminatum* populations from the Nullarbor region of South Australia (Fuentes-Cross 2016). Significant genetic structuring and admixed populations were found in the Nullarbor region, indicating opportunities for long-distance gene flow, rather than isolation by natural landscape barriers, was the primary driver of detected genetic variation in the desert landscape.

Importantly, our study revealed that while genetic diversity and low to negligible levels of inbreeding were comparable across most sites, the study system is characterised by directional gene flow from the Northern Territory southwards into South Australia. This directional movement of genes is consistent with the scenario that the Desert Quandong in the northern extent of its range comprises a series of remnant populations that historically likely were more numerous and widespread. Importantly, these NT populations contain important reservoirs of extant genetic diversity and geographic occupancy contributing significantly to ongoing and future persistence of the species.

The relative sampling effort of South Australian populations makes inferences about southern population processes tentative as there was greater relative geographic distance between sampled sites and isolation between these sites by landscape features. There would have been many other stands of Desert Quandong occupying locations between these sites. The detection of low population structuring between the majority of sites sampled from South Australia but strong genetic structuring with

the River Murray district is likely to reflect the relative high geographic distance, and the presence of geographic landscape barriers (drainage basins), between the River Murray collections compared to other sites. It would be expected that the extent of connectivity and gene sharing between stands from different basins would be lower across than within basins resulting in greater differentiation.

Patterns of regional population differentiation and negligible inbreeding have also been reported from the monsoonal dry tropics of northern Queensland in a related species, *Santalum lanceolatum*, (Brunton et al. 2021), although the drivers of differentiation were attributed to relatively recent impacts of disturbance by human activities resulting in reduced natural recruitment rather than landscape or environmental factors.

The molecular markers developed and applied in this study provide a significant, robust genetic tool to support future analysis and monitoring of wild populations of the Desert Quandong. Application of these genetic resources can track changes in genetic diversity and determine the population relationships among priority stands of plants on Parks.

This study provides supporting evidence that the northern populations may be eligible to be listed as nationally threatened. The significant reduction or loss of northern genetic lineages of Desert Quandong under continuing threatening processes presents a potentially significant risk to the ongoing maintenance of this nationally important widespread species. The distinct, extant genetic lineages have importance as genetic diversity sources for more southern sites. Our increased knowledge of genetic relationships among stands will support us to prioritise management as well as implement recovery actions relating to seed sourcing, propagation and augmentation. Based on the genetic data Mulga Park could provide a useful source of seed for recovery projects such as for propagule production to be translocated to Uluru Kata-Tjuta National Park. This is due to the relatively large population size, the presence of many non-clonal stands and a genetic diversity profile that is widespread across the region. Watarrka National Park should be considered as a critical source site for recovery efforts to maximise genetic representativeness and support sustainable use as the desired outcome, for example in ensuring the genetic diversity from both northern lineages are represented in an orchard establishment.

Implementation of tailored management actions is needed to address the particular threats faced by Desert Quandong at Watarrka National Park and Mulga Park. Assessments of stand health and demographic monitoring have demonstrated that the Mulga Park stands are in very poor condition due to recent severe camel damage. There is a significant risk that the cultural and ecological values of this Desert Quandong site will be lost and the stands severely degraded (Nano et al. 2020). Immediate intervention to protect stands from browsing and damage from introduced herbivores is needed to remove this threat to support Mulga Park as a viable population on pastoral land. At Watarrka National Park, uncontrolled and repeated

wildfire has had a major impact on the size and demography of Desert Quandong stands (Nano 2016; Nano et al. 2020).The likelihood of fire survival is related to landscape position, with survival consistently higher on the rocky sandstone plateau and relatively low and variable in the sandy creek lines on the plain (Nano 2016). Introduced grass invasion including by Buffel grass and associated species likely increase fuel loads and activity and is also a recognised potential threat to the Desert Quandong. Implementation of integrated fire planning and management at Watarrka including protection of stands from repeated fire, along with ongoing monitoring and management of invasive introduced grasses, should be a high priority action for maintenance of Desert Quandong and all biodiversity values in the park.

Enhancing our ability to identify and select Desert Quandong stands and identifying genetic connectivity pathways has proven to be valuable to more effective decision-making for this species. Research that improves the coverage of genetic diversity assessment of stands in northern South Australia and the Northern Territory would be of considerable value. This would support efforts to preserve northern genetic lineages of Desert Quandong and inform future national conservation and recovery planning for the species.

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