Evaluating restoration outcomes through assessment of pollen dispersal, mating system and genetic diversity

Running Head: Genetic function in Hakea restoration

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Keywords: frog hakea, mating system, pollen dispersal, pollen immigration, restoration success criteria, Western Australia
Abstract

Ecological genetics can provide a novel contribution to assessing the achievement of restoration objectives. We used paternity assignment to infer realized pollen dispersal within, and pollen immigration into, a restoration population of Hakea nitida, a common near-coastal shrub or small tree in southwest Australia. We compared mating system parameters and genetic diversity with a nearby remnant reference population and assessed genetic divergence among the restoration and reference population. We found realized pollen dispersal events closely tracked the frequency distributions of the distances between all plants within the restoration focus area. Mean realized pollen dispersal distance (359 m) approached the mean of the distances between all plants (407 m), far exceeding mean nearest neighbour distance (12 m). Maximum realized pollen dispersal distance (869 m) approached the maximum distance between all plants in the study area (1033 m). Pollen immigration into the restoration study area was limited (4%). The mating system revealed moderate outcrossing rates ($t_m=0.861$ restoration and $t_m=0.745$ reference population), with significant and similar biparental inbreeding ($t_m-t_s=0.180$, $t_m-t_s=0.186$) but greater correlated paternity ($r_{pm}$) in the restoration (0.519) than in the reference (0.188) population. Genetic divergence among the restoration and reference remnant population was moderate ($F_{ST}=0.094$, $D_{ST}=0.239$). Patterns of pollen dispersal and mating system parameters imply the attraction of pollinators within the restoration population as a key factor in progressing towards establishment of self-sustaining populations.
Implications for Practice

- Ecological genetic studies should be considered in order to assess the degree of functional connectivity, effectiveness of animal pollinators in maintaining mating systems, and the appropriateness of the size and type of seed collections, for restoration populations.

- Effective pollinator services may be achieved in restoration populations of animal-pollinated species even when effective population sizes of restoration and local remnant populations are small and plant densities are low.

- Accurate seed sourcing records for restoration activities are important in interpreting levels of genetic diversity captured in restoration populations and levels of genetic divergence among restoration and local remnant populations.

Introduction

Objectives for restoration typically focus on structural goals, such as number of individuals surviving, species established or percentage of ground cover after a given time frame (Pavlik 1996). Increasingly, the practice of ecological restoration addresses objectives based on more functional requirements for population persistence (Forup et al. 2008; Young et al. 2019), such as the establishment of populations that are self-sustaining, that become integrated into the broader landscape, that are adaptive and resilient in the long-term, and that contribute functionally to effective ecosystem services (McDonald et al. 2016b; SERA 2017). This recognition has encouraged the development of post implementation and long-term, empirical evaluations of how well restoration activities have met their objectives (Ruiz-Jaen & Aide 2005; Aavik & Helm 2017; SERA 2017). In this context, the field of ecological genetics can provide a novel contribution to the assessment of restoration objectives relating to
functionality, integration of restoration populations into the broader landscape, and likely persistence (Young et al. 2005; Monks et al. 2012; Ritchie & Kraus, 2012; Frick et al. 2014; McDonald et al. 2016a; Aavik & Helm 2017).

Attracting pollinator services is a critical aspect of restoration that is required for population persistence in the short and long-term (Dixon 2009). Pollinators are required for seed set, maintain mating systems, and contribute to reproductive potential and maintenance of genetic diversity in restoration populations of animal-pollinated plant species (Dixon 2009). This makes the attraction of pollinator services an essential objective for most restoration activities. For animal-pollinated plant species, pollinator diversity, abundance and activity will affect patterns of fine-scale pollen dispersal and mating within populations. If pollinator assemblage, abundance or activity is suboptimal, restoration populations of predominantly outcrossing but self-compatible animal-pollinated plant species may experience limited pollen diversity and little fine-scale pollen dispersal. This may lead to reduced levels of outcrossing, and reduced seed set or seedling fitness (Krauss et al. 2007; Yates et al. 2007; Gibson et al. 2012; Llorens et al. 2013) associated with inbreeding depression (Fenster & Dudash 1994; Lesica & Allendorf 1999).

Attracting animal pollinator services in restoration populations may be particularly challenging in highly fragmented landscapes. For animal-pollinated plant species, the attraction of pollinator services in restoration populations would be expected to depend on proximity to remnant vegetation that supports pollinators, the dispersal ability of pollinators across altered habitat matrices, and the ability of pollinators to potentially establish within the population (Forup et al. 2008; Dixon 2009; Menz et al. 2011). Assessing restoration
objectives in terms of pollinator services will require an understanding of pollen dispersal in fragmented landscapes and the capacity of pollinators for dispersal and migration into, and recolonization of, restoration populations (Menz et al. 2011). Genetic assessments of pollen dispersal via paternity analysis and mating system parameters may also inform on achievement of restoration objectives related to reconnected habitat and ecosystem function.

Restoration practice often seeks to use germplasm that is genetically diverse and of the local provenance (Millar & Libby 1989; Mortlock 1999; Broadhurst et al. 2008). Others maximize genetic diversity and evolutionary potential through admixture or climate adjusted provenance strategies (Breed et al. 2013; Prober et al. 2015) considering potential outbreeding depression and negative ecological interactions such as invasiveness, hybridisation and displacement of local form (Hufford & Mazer 2003; Broadhurst et al. 2008; Byrne et al. 2011). Genetic diversity is important for the maintenance of outcrossing and the prevention of potential inbreeding depression (Albrecht & Maschinski 2012) in self-compatible plant species. It assists in maintaining population fitness (Fenster & Dudash 1994; Lesica & Allendorf 1999) for such species, and has been linked to increased productivity (Bischoff et al. 2010) and the long-term resilience of plant populations in general (Hughes & Stochowicz 2004; Reusch et al. 2005; Johnson et al. 2006). Genetic diversity in restored populations will reflect the collection strategy used and the size and diversity of the source population(s) from which seed or other propagules are collected (Krauss et al. 2002; Coates & Byrne 2005; Krauss et al. 2007).
Restoration sites in the Gondwana Link program in south-western Australia provide excellent study areas in which to conduct ecological genetic assessments of restoration for a range of plant species with varying life history traits (Fernandes 2016; Millar et al. 2019a; Millar et al. 2019b). The Southwest Australian Floristic Region and biodiversity hotspot (SWAFR, Hopper & Gioia 2004, Gioia & Hopper 2017) is a landscape where native vegetation has been largely cleared with numerous often small fragmented remnants remaining (Beard 1999). The Gondwana Link program is a non-profit, private sector, collaborative organisation leading an ambitious large-scale conservation and restoration initiative in the region (Bradby 2013). This program aims to provide reconnected habitat in which ecosystem function and biodiversity are restored and maintained (Bradby et al. 2016).

Here, we evaluate progress towards providing reconnected habitat in which ecosystem function and biodiversity are restored and maintained by assessing fine-scale intrapopulation pollen dispersal, and genetic connectivity via pollen immigration, for a Gondwana Link restoration population of the animal-pollinated *Hakea nitida* R. Br. (Proteaceae). We compared mating system parameters in a restoration population to those of a nearby remnant, the recorded seed source population. In addition, we compared levels of genetic diversity in the restoration and nearby remnant population and estimated the degree of genetic divergence. If widely recognised guidelines (e.g. Gann et al. 2019) for seed collections were followed, levels of genetic diversity within the restoration and remnant reference study populations should be largely equivalent, and levels of genetic divergence between the restoration and remnant study populations should be low.
Specifically we evaluate the following expectations of reproductive functionality for a restoration population of *H. nitida*; (i) the frequency distribution of realized mating will closely track the frequency distribution of the inter-mate distances between plants reflecting random pollen dispersal; (ii) there will be pollen immigration from outside the restoration study area, and; (iii) mating system parameters in the restoration population will be largely equivalent to those in the remnant reference population. We also expect that (iv) genetic diversity within the restoration population will be equivalent to that of the remnant reference population, and; (v) genetic divergence between the restoration and known seed source remnant population will be negligible.

**Methods**

**Study species**

*Hakea nitida*, also known as the frog hakea, or shining hakea, is a spreading, many stemmed shrub or small tree, generally 1-3 m high with shiny leaves with length up to 9 cm (ABRS & CSIRO 1999). White, cream or yellow, fragrant, dome shaped, floral clusters are produced from July to September. Plants are visited by a range of bird species but are likely to be pollinated largely by generalist insects given the flower structure. Details of the mating system are unknown. However, analysis in another *Hakea* species showed a highly outcrossed system with mating between relatives but limited selfing, suggesting a mechanism to prevent self-pollination and/or selection against relatively inbred offspring (Sampson et al. 2016). Plants occur in grey, yellow or brown sand, loam, or lateritic clay, in coastal sand dunes and quartzite and granite hills along the southern coast of Western Australia, from Albany to east of Israelite Bay (Western Australian Herbarium 1998). Seeds are borne in woody capsules with up to two seeds per capsule. Seeds have a wing broadly down one side suggesting wind dispersal and
are up to 24 mm long (including wing). Seeds of several hakeas are known to have been used by Noongar people to form an edible paste (L. Knapp 2017, personal communication). Hence, local dispersal by Aboriginal people may have occurred over long time frames. Plants are non-lignotuberous, so they are likely to be killed by fire (Western Australian Herbarium 1998).

Study populations and sampling

The restoration study site is located between the Fitzgerald River and the Stirling Range National Parks in the SWAFR (Figure 1). Remnant vegetation largely persists along heavily disturbed roadsides in this region, although there are also a few nature reserves and several private property areas consisting of relatively intact remnant vegetation. The Peniup Creek Reserve is a property involved in the large-scale Gondwana Link restoration project. Plantings of native species including *H. nitida* commenced at Peniup in 2008 (Jonson 2010). Seed for *H. nitida* restoration is recorded as being sourced from a small (~1 ha) remnant population less than 2 km away. This natural population was sampled as the reference population for this study as it is the closest recorded remnant population and the recorded seed source. The next nearest recorded remnant population of *H. nitida* is located ~28km from the restoration population and ~30km from this reference population. The reference population comprised 23 plants when sampled. Population density was estimated at 23 plants ha⁻¹. Leaf material was sampled in 2016 from 20 plants of *H. nitida* at the remnant population. Sufficient capsules were present on ten plants from which seed was sampled.

Proteaceous species including *H. nitida* were planted in ‘nodes’ or spatially aggregated clumps of seedlings ranging from 10 to 50 individuals at the Peniup Creek Reserve (Jonson 2010). The premise behind this was the efficient use of a limited quantity of seed by providing
concentrated flowering resources that would attract pollinators and enhance pollen dispersal (Jonson 2010). Additional plants were also established at random throughout the area. Leaf material was sampled from 172 plants in the restoration population, representing all plants that could be found within an approximately 90 ha study area of restoration vegetation (density of ~2 plants ha\(^{-1}\) but highly variable over the study area). Other plants may have been present outside this study area as other parts of the 2406 ha reserve have also been planted with *H. nitida*, and these areas were not exhaustively surveyed. Seeds, presumably resulting from flowering in 2015 were sampled from the 27 plants within the study area that had capsules. All sampled plants were tagged and labelled, and spatial coordinates were recorded with a Global Positioning Satellite system (Garmin, Olathe, USA).

Leaf material was freeze dried and stored on silica. Capsules from each mother plant were stored separately. Woody capsules were dried at 15°C until they opened. Seeds were extracted from fruit and up to 30 seed from each mother plant (numbers available varied) were germinated on agar at 18°C. Seedlings were freeze dried and stored on silica when cotyledons and the first true leaves had emerged.

Library construction and genotyping

DNA extraction was conducted on leaf and seedling material following Doyle and Doyle (Doyle & Doyle 1987) with the addition of PVP-40T polyvinyl pyrrolidine to the extraction buffer and two chloroform extraction steps. Genomic DNA from one adult individual was sequenced on a MiSeq (Illumina Inc, San Diego, USA), at Monash University, Malaysia. DNA was quantified and randomly sheared into fragments of ~500 bp on a Covaris ultrasonicator (Covaris Inc, Woburn, USA) with library preparation using NEBNext Ultra DNA Library Prep kit for Illumina
(New England BioLabs Inc, Ipswich, USA), library target enrichment and quality control on a Tapestation (Agilent, Santa Clara, USA), denovo assembly, and identification of microsatellite regions and primer pair construction using QDD based on the authors suggestions (Meglécz et al. 2010). Design A (Meglécz et al. 2010) primers were filtered to remove AT motifs and target repeat number lengths less than five. This resulted in 64 potentially suitable primer pairs.

Amplification and polymorphism of microsatellite regions was evaluated on eight randomly selected individuals. Each PCR consisted of 0.5 µl DNA, 2.75 µl H2O, 0.45 µl 3 µM MgCl₂, 1.125 µl each of 2 µM forward and reverse primer, 1.5 µl 5 x PCR buffer and 0.05 µl taq polymerase for a final volume of 7.5 µl. The PCR program consisted of 96 °C for 2 min, 30 cycles consisting of 95 °C for 30 sec, 56 °C for 30 sec and 72 °C for 30 sec, and a final extension of 72 °C for 5 min. Twelve of 60 tested primer pairs produced reliable and polymorphic products (Hn03, Hn06, Hn12, Hn13, Hn19, Hn22, Hn24, Hn33, Hn47, Hn52, Hn55, Hn58, Table 1). Forward primers were made with a fluorescent dye label of the G5 label set (FAM, VIC, NED or PET). A primer master mix was made using 100 mM forward and reverse primers at varying concentrations depending on the dye label (FAM, VIC, NED or PET, Table 1). Microsatellite regions were amplified for all individuals by PCR in a Qiagen® Multiplex kit (Agilent Inc). Each PCR consisted of 1.0 µl DNA, 2.0 µl H2O, 0.75 µl of primer master mix and 3.75 µl of Q mix (Qiagen® Multiplex kit) for a final volume of 7.5 µl. The PCR program consisted of 95 °C for 15 sec, 35 cycles consisting of 94 °C for 30 sec, 60 °C for 90 sec and 72 °C for 60 sec, and final extensions of 72 °C for 30 sec and 25 °C for 60 sec. One µl of each of PCR product was added to 12 µl of GeneScan™ LIZ®500(-250) size standard (Applied Biosystems, Waltham, USA)/formamide, and fragments were visualised via automated fluorescent scanning.
detection using an Applied Biosystems 3730 DNA Analyser. Genotypes were scored using GeneMapper v3.7 (Applied Biosystems). Allele bins were manually assigned and automatically checked. When necessary, alleles were manually adjusted. True positive controls are not available for de-novo studies such as these; however, we re-amplified samples that did not amplify well, at least once, along with positively amplifying samples.

Individual loci were tested for departure from Hardy-Weinberg equilibrium and locus pairs tested for linkage disequilibrium (LD) for adult plants in all populations, using exact tests as implemented in GENEPOP v4.0 (Raymond & Rousset 1995). Sequential Bonferroni corrections were applied. Presence of null alleles was assessed with the MICRO-CHECKER v2.2.3 software program (Oosterhout et al. 2004) for all adult individuals and frequencies estimated using the Brookfield Estimator 1 (Brookfield, 1996) assuming no null homozygotes.

**Pollen dispersal and immigration**

A total of 738 progeny collected from the restoration population were genotyped for estimation of pollen dispersal via paternity assignment and pollen immigration via paternity exclusion. Genotypic data for progeny from known mother plants was combined with known genotypes of all plants in the restoration population study area and assessed for paternity analysis. We considered all plants within the restoration population study area as potential fathers since all plants were mature and presumably capable of producing pollen, although not all were observed flowering at the time of sampling.

Paternity assignment analysis was conducted using CERVUS (Marshall et al. 1998). This program finds optimal progeny-mother-father parent trios and conducts fractional
assignment of paternity using a maximum likelihood-based approach for statistical evaluation of the matches (Marshall et al. 1998). We ran the program for each population with simulation of 10,000 progeny, a known number of potential male parents (i.e., the total number of plants within the focused study area), 96% of potential male parents genotyped, and an error rate of 1%. Critical Delta criteria (defined as the LOD (the natural log of the overall likelihood ratio) scores for the most likely paternal parent) were obtained from simulations and used as a criterion for assignment of parentage. We compared trio LOD scores to assign most likely paternal parents at a strict (95%) confidence level, a relaxed (80%) confidence level, and at less than 80% confidence. We also assessed whether there was more than one equally likely potential father within the population (equal positive LOD scores for more than one most likely paternal parent) or whether there was no potential paternal parent within the population (i.e., a result of pollen immigration, negative LOD score). Due to the potential for selfing, we allowed known mothers to be potential male parents and considered all most likely fathers assigned with confidence of 80% or more within a population as the pollen donor. Further analysis was conducted for progeny assigned a most likely father with confidence of 80% or more.

The percentage of selfed progeny was determined via paternity assignment as the percentage of progeny that were assigned their female parent as the pollen donor. We calculated the frequency distributions of the geographic distances between all pairs of plants. Mean nearest neighbour distances within the study plot were also calculated by taking the mean for all plants of the geographic distance between each plant and its nearest neighbour. Realised pollen dispersal distances were calculated as the geographic distance between maternal plants and the assigned pollen donor. Pollen dispersal distances were sorted into 18 x 50 m
categories and the frequency distributions of the proportions of realised pollen dispersal events in each distance category and the frequency distributions of the proportions of geographic distances between all plants in each distance category were plotted. We used a Kolmogorov-Smirnov test to assess significant differences in the frequency distributions of the proportions of realised pollen dispersal events and the frequency distributions of the geographic distances between all plants. This was conducted via a two-sample test using the SAS v9.3 software (SAS Institute Inc 2011). The mean number of unique pollen donors per maternal plant was calculated \( (N_p) \) over the 27 maternal plants. The mean percentage of unique pollen donors for the number of progeny assigned a most likely father per maternal plant was also calculated.

Paternity exclusion was used to estimate pollen immigration (pollen flow into the restored study area) using the Pollen Flow program (Slavov et al. 2004). In this approach the progeny genotypes are compared to those of potential male parents in the local population (in this case all genotyped plants within the ~90 ha study area). Incompatible progeny with multilocus genotype mismatches are assumed to result from immigration from outside this area (Slavov et al. 2004). The program also estimates cryptic gene flow i.e. the proportion of pollen haplotypes in a background population that are identical to haplotypes that could be produced in the local population. This produces a more conservative estimate of immigration. Pollen Flow allows for mistyping due to null alleles, mutations and detection errors. We used the diploid sampling scheme and tested for immigration at a conservative level requiring a minimum of three progeny/male parent mismatches for exclusion.
Mating system

Mating system parameters for each population were analyzed under both the mixed and correlated mating models using MLTR 3.4 (Ritland & Jain 1981; Ritland 2002). The expectation-maximization (EM) method was used to estimate the means and standard deviations of the following: the multilocus outcrossing rate ($t_m$) and single-locus outcrossing rate ($t_s$), estimated rate of biparental mating ($t_m-t_s$), correlation of selfing or outcrossing among maternal plants ($r_s$), correlation of selfing among loci ($r_{sl}$), and multi-locus correlation of paternal plants ($r_{pm}$, Ritland, 2002). The standard deviation of each estimate was based on 1000 bootstraps. An effective number of pollen donors (Nep) was determined as $1/r_{pm}$.

Genetic diversity and divergence

Parameters including the number of individuals genotyped ($N$), and estimates of nuclear microsatellite diversity (the mean number of alleles per locus ($N_a$), the effective number of alleles ($N_e$), the number of private alleles ($P$), the number of private alleles after rarefaction ($P_r$), the proportion of polymorphic loci ($P_o$), expected ($H_e$) and observed ($H_o$) heterozygosity and the Fixation index ($F$) were calculated for 12 loci using GenAlEx v6.5 (Peakall & Smouse 2012). The rarefied number of alleles ($N_{ar}$) which accounts for differences in samples sizes, was calculated using HPRare (Kalinowski 2005) assuming a minimum of 30 alleles for adult cohorts and 32 for progeny cohorts. Estimates were obtained for adult and progeny cohorts separately.

Levels of fixation and allelic divergence for adult individuals between populations were assessed using pairwise Wrights’ $F_{ST}$ and Josts’ $D_{ST}$ values respectively, with values obtained via GenAlEx. Genetic structure was further investigated for adult individuals within the
restoration population using the Bayesian assignment approach in STRUCTURE v2.3.2.1 (Pritchard et al. 2000). Analysis used the admixture ancestry model with the assumption of correlated allele frequencies amongst samples (Falush et al., 2003). A burn-in period of 10,000 was applied with 100,000 MCMC replications to assess the optimal number of clusters (K) for K values ranging from 1 to 10, with 10 iterations of each K value. The optimal K value was determined with STRUCTURE HARVESTER v.0.6.93 (Earl and VonHoldt, 2012) by assessing the ad hoc quantity LnP(d), which is the log of the probability of the data calculated in STRUCTURE and the variation in LnP(d) DeltaK following the methods of Evanno et al. (2005). Mean permuted proportion of membership (Q) values for all populations were graphed.

Results

Utility of loci

Several loci showed a departure from Hardy-Weinberg equilibrium with significant deficits of heterozygotes at the Peniup restoration population (loci Hn03, Hn06, Hn12, Hn19, Hn33, Hn47, Hn55 and Hn58) and at the Peniup remnant population (locus Hn24). Ten locus pairs showed significant LD but only in the restoration population. Possible presence of null alleles was detected for eight loci at the Peniup restoration population and two loci at the Peniup remnant population. Calculated frequencies of null alleles are provided as Supplementary Material (Table S1). Null alleles are common in microsatellite data sets and may be affecting the linkage disequilibrium detected at loci. However, calculation of and correction for of error rates requires the use of a reference genome which is not available for this species. As a result, we retained all loci for the study.
Pollen dispersal and immigration

The probability that the set of loci used in this study will exclude an unrelated candidate male parent from paternity of an arbitrary progeny when the genotype of the mother is known was 0.9910. Paternity assignment resulted in 8.4% of analysed progeny being assigned a most likely father within the restoration site with 95% confidence or more, 27.5% with 80% to 95% confidence, and 21.8% with less than 80% confidence. The remaining 42.3% of progeny were not assigned fathers meaning either there was insufficient power in the markers to assign a single most likely father or the pollen donor originated from outside the restored study area.

The mean (maternal plant) estimates of cryptic pollen dispersal (assignment of a genotyped plant within the stand as the pollen donor when the true pollen donor is outside the stand) determined by paternity exclusion was low, 1.3%. This indicates a low number of false positive paternal assignments. The estimate of pollen immigration into the restoration study area was also low 3.97% ± 0.026.

The frequency distribution of the proportion of realised pollen dispersal events in each distance category (for progeny assigned a most likely father within the restoration site with 80% confidence or more [265 progeny, 35.9% of progeny genotyped]) closely tracked the frequency distributions of the proportion of distances in each distance category between all plants (Figure 2). There was no statistically significant difference between the two dispersal frequency patterns ($p = 0.9829$). The percentage of selfed progeny (i.e. the percentage of progeny that were assigned their female parent as the most likely male parent from within the genotyped study area with confidence of 80% or more) was 1.1%. The maximum realised pollen dispersal distance inferred from paternity assignment of 869 m was similar to the maximum potential pollen dispersal distance of 1033 m. The mean realised pollen dispersal
distance (359 m +/- 13.8 m) was also similar to mean potential pollen dispersal distance of
407 m +/- 3.4 m and far exceeded the mean nearest neighbour distance within the study area
of 11.7 +/- 1.2 m. The geographic distance class with the greatest proportion of pollen pairs
and number of pollen dispersal events was 350-400 m. For the 265 progeny assigned a father
with confidence of 80% or more, the mean number of unique pollen donors per maternal
plant averaged 6.9 +/- 0.8. When unweighted for the number of progeny assigned a most likely
father, the percentage of progeny assigned a unique most likely father per maternal plant
averaged 79.53%.

Mating system
The overall multilocus estimate of outcrossing rate was significantly lower than one (mean \( t_m \)
= 0.803 +/- 0.058, Table 2). The estimate of biparental inbreeding was significantly greater
than zero for each population and for both populations overall (mean \( t_m - t_s = 0.183 +/- 0.003, \)
Table 2). Estimates of the degree of correlation in selfing among loci suggest that all apparent
selfing (1-\( r_s \)) is due to mating between related individuals in the remnant population.
Multilocus correlated paternity varied and was significantly greater for the restoration
population compared to the remnant (\( r_{pm} = 0.519 +/- 0.112 \) and \( 0.188 +/- 0.047, \) Table 2). The
estimated number of effective pollen donors over all maternal plants per population was
correspondingly lower for the restoration population compared to the remnant.

Genetic diversity and divergence
Diversity values were generally higher in the restoration population than in the remnant
population of \( H. \) nitida for both adult and progeny cohorts (Table 3). The number of alleles
and number of private alleles after rarefaction were significantly greater in the restoration
adults than the remnant population adults. Fixation indices were positive in both populations and cohorts, and significantly greater than zero for the restoration adults, and both remnant and restoration progeny (Table 3), reflecting an excess of homozygotes or inbreeding and retention of inbred individuals in both adult and progeny cohorts.

The remnant and restoration population adults showed moderate levels of genetic fixation ($F_{ST} = 0.094, +\text{-}0.028, p= 0.001$), and a moderate degree of allelic divergence ($D_{ST} = 0.239 +\text{-}0.083, p = 0.001$). STRUCTURE analyses optimally identified two genetic clusters via the maximum Delta K (Figure S1) with limited admixture between the two clusters (Figure 3). The first cluster (cluster 1, Figure 3) comprised the reference remnant population individuals and 45 of the restoration individuals. The remaining 127 restoration individuals were placed in a separate cluster (cluster 2, Figure 3).

**Discussion**

Genetic diversity and pollinators are important for ecological genetic functionality in restoration populations of animal-pollinated plants. Our assessment of these factors in a restoration population of *H. nitida* showed random pollen dispersal and mating system parameters that were largely equivalent to those in the remnant reference population. This indicates effective pollinator services within the population, although pollen immigration into the restoration study area from nearby restoration or remnant plants appeared limited. Genetic diversity captured within the restoration population was greater than that of the remnant reference population and there was an unexpectedly high degree of genetic divergence among the restoration and known seed source remnant population with a second, unrecorded seed source highly likely. Overall, our results suggest levels of genetic diversity
that indicate progress toward restoration objectives and effective pollination services within
the restoration population.

**Pollen dispersal**

We found a pattern of fine-scale pollen dispersal that closely tracked the spatial distribution
of founder individuals in the restoration population of *H. nitida*, suggesting plant distribution
influences pollinator movement at this site. Overall, the mean distance of realised pollen
movement of *H. nitida* greatly exceeded the mean nearest neighbour distances within the
study area. This contrasts with historical optimal foraging theory that predicts an animal will
adopt a foraging strategy that provides the most energy benefit for the lowest cost (Pyke
1978), and theoretical expectations of a leptokurtic pattern of pollen dispersal, characterised
by nearest neighbour mating with a predominance of short range pollination distances for
animal-pollinated plant species (Levin & Kerster, 1974). Instead, pollen dispersal was
characterised by predominantly mid-distance pollination events, with a fat tail of long-
distance dispersal events. Interestingly, the extent of pollen dispersal was greater than that
in natural populations of *H. oldfieldii* that showed intermediate pollination between nearest
neighbour and random pollen dispersal (Sampson et al., 2016). Combined with a high
outcrossing rate, a pattern of random realised pollen dispersal suggests the attraction of
effective pollinators for the restoration population of *H. nitida*. The specific pollinators of *H.
nitida* are unknown, although floral characteristics suggest predominantly insect dispersal.

Pollen immigration from outside the study area was detected at a low rate for *H. nitida*. The
source of immigrant pollen may have been un genotyped *H. nitida* individuals within the
restoration vegetation but outside the focal study area, or a natural remnant population. The
nearest known natural remnant is the reference population located approximately 2 km from
the restoration site. Although limited in amount, pollen immigration into the study area may
well have occurred over such a distance, as pollen dispersal over this distance has been
documented in another *Hakea* species (Sampson et al., 2016). Generalist insect pollinators
are known to be capable of extensive pollen dispersal in many fragmented landscapes. For
example, pollen dispersal distances of up to 1566 m have been detected for *Acacia saligna* in
a fragmented agricultural area of the SWAFR (Millar et al. 2008; Millar et al. 2014), over 1870
m for *Acacia woodmaniorum* across ironstone inselberg outcrops in the semi-arid zone of
Western Australia (Millar et al. 2014), and over distances of several kilometers in eucalypt
species of other fragmented Australian landscapes (Byrne et al. 2008; Sampson & Byrne 2008;
Ottewell et al. 2009; Broadhurst 2013). Similar long pollen immigration distances have been
detected for woody perennials in fragmented tropical dry forests of the Honduran Pacific
coastal plain (White et al. 2002), both fragmented and pristine Amazonian rainforest (Dick et
al. 2003), the neotropics of Panama (Nason et al. 1998), and in shade coffee farms of
neotropical southern Mexico (Jha & Dick 2010), with exceptional wind assisted insect
dispersal over 160 km detected in the desert gravel plains of southern Africa (Ahmed et al.
2009). Although limited, the detection of pollen immigration into the study area suggests a
degree of genetic connectivity for the restoration population at the greater landscape scale.

**Mating system**

Mixed mating with high rates of outcrossing and a degree of selfing largely due to mating
among relatives was observed in both the remnant and restoration populations of *H. nitida*.
Similar mixed mating systems have been found for other Proteaceae including *Hakea laurina*
(Fernandes, 2016), and some *Banksia* species (Sampson et al. 1994; Wooller & Wooller 2002;
Coates et al. 2013), although, mating system analysis in another *Hakea* species showed high outcrossing rates indicative of self-incompatibility (Sampson et al. 2016). Along with random patterns of pollen dispersal, largely equivalent mating system parameters in the restoration and reference remnant population provide further support that pollinator services have been attracted to the restoration population of *H. nitida*. The result is consistent with other studies that have inferred the rapid attraction of pollinator services in restoration populations via assessment of the mating system. These include the insect-pollinated *Acacia cyclops* (Millar et al. 2019a) and *Melaleuca acuminata* (Millar et al. 2019b), and the animal-pollinated *H. laurina* (Fernandes, 2016) and *Banksia media* (Millar et al. 2020), at this and other Gondwana Link restoration sites. It has also been shown for the animal-pollinated *Banksia attenuata* (Ritchie & Krauss 2012) and vertebrate-pollinated *B. menziesii* (Frick et al. 2014) in the SWAFR, and insect-pollinated *Eucalyptus melliodora* in eastern Australia (Broadhurst 2013).

Equivalency in outcrossing rates for the restoration and reference population suggest that progress towards restoration objectives is being made.

High levels of outcrossing should act to maintain heterozygosity in subsequent seed and progeny of plant species. The lower levels of observed heterozygosity compared to expected heterozygosity, and positive estimates of fixation indices indicate some form of inbreeding in adults of the restoration population and progeny cohorts of both remnant and restoration populations of *H. nitida*. Although outcrossing estimates indicate that some self-pollination is taking place, low estimated correlation of selfing among loci suggests that the majority of selfing is due to mating among closely related individuals in the remnant population (Ritland 2002). This form of inbreeding typically occurs with small effective population size and limited mate availability. Limited mate availability is also evidenced by the low pollen diversity...
observed in both restoration and remnant populations of *H. nitida* in this study. These measures were generally lower than that reported in other species, such as for remnant populations of *H. oldfieldii* (Sampson et al. 2016), both remnant and restoration populations of the bird- and insect-pollinated *H. laurina* in the same landscape (Fernandes, 2016), both remnant and restoration populations of the animal-pollinated *B. attenuata* (Ritchie & Krauss 2012), and in remnant populations of the mammal-pollinated *Banksia nivea* (Thavornkanlapachai et al. 2018), the animal-pollinated *Banksia sphaerocarpa* (Llorens et al. 2012) and *Banksia hookeriana* (Krauss et al. 2009).

Significant inbreeding and low pollen diversity may not be unexpected for both restoration and remnant populations of *H. nitida*. While spatial genetic structuring and gene flow via pollen immigration was not assessed in the remnant reference population, a degree of genetic structure and small effective population size may be expected. Local remnant populations of *H. nitida* tend to be small (a few tens of plants), and although mass flowering, flower to seed conversion is typically limited for Proteaceae, and primary seed dispersal is typically limited in *Hakea* (5-20 m, Groom 2010); traits that would encourage genetic structuring. Spatial genetic structuring would not typically be expected in founder individuals of restoration populations comprised of a large number of seed sourced from genetically diverse seed sources, due to random spatial dispersal founders at establishment. Significant inbreeding and significantly lower pollen diversity for maternal plants of the restoration compared to the remnant population may suggest collections from a limited number of maternal plants within seed source populations. This may also be affected by the spatial aggregation of individuals of *H. nitida* in nodes at the Peniup restoration site. A similar pattern of significant inbreeding and lower pollen diversity has been detected for *H. laurina* which is
also planted in nodes at the Peniup restoration site (Fernandes 2016). In contrast, limited inbreeding and pollen loads with high diversity have been identified in a stand of *A. saligna* where individuals were planted at evenly spaced distances in a grid like pattern (Millar et al. 2008), and in *B. media* restoration where founders were also planted in an evenly spaced grid like pattern (Millar et al. 2020). Unlike *Hakea*, which are mass flowering with floral displays lasting for a few weeks, *A. saligna* flowers opportunistically throughout the year and *Banksia* also tend to flower for many months of the year, which would provide greater temporal opportunity for more diverse pollen pools. Inbreeding and low pollen diversity in the *H. nitida* restoration may also be affected by a low number of flowering plants.

Inbreeding and limited pollen diversity for the year of seed production sampled here (2015) is also likely related to a limited number of flowering individuals and low effective population density of both the restoration and remnant populations. The total number of plants that flowered in 2015 in each population is not known, although seed was observed on only a limited number of plants in both the restoration and remnant populations at the time of sampling for this study (2016). Flowering observations for the year of sampling also suggest limited mate availability due to limited flowering or limited temporal overlap in flowering. Aspects of flowering phenology and pollinating fauna are known to vary greatly among populations and over time (Brunet & Sweet 2006; Kameyama & Kudo 2009; Karron & Mitchel 2012), and plant mating systems also vary spatially (Whitehead et al. 2018) and temporally (Murawski & Hamrick 1991; Nason & Hamrick 1997; Butcher et al. 2011; Coates et al. 2013). This makes it difficult to generalise regarding ecological drivers of limited pollen diversity in the study populations of *H. nitida*. Our findings of random spatial pollen dispersal and high outcrossing suggest that limited mate availability may be more related to aspects of floral
phenology and fecundity than limitations to pollen dispersal distances for *H. nitida*. Ecological assessments of flowering phenology and fecundity would help to elucidate the drivers of inbreeding and low pollen diversity in these populations.

It is not known to what extent *H. nitida* may be affected by inbreeding depression, although species with largely outcrossed mating systems experiencing limitations to mating are expected to suffer negative genetic and demographic consequences due to inbreeding depression over time (Montalvo et al. 1997). If limited fecundity and mate availability persists and recruitment occurs at some future time in the restoration population, spatial genetic structure may develop. In conjunction with limited seed dispersal, this may result in greater levels of inbreeding and associated negative genetic and demographic effects in the restoration population. Alternatively, *H. nitida*, like *H. oldfieldii*, may be tolerant of inbreeding through purging of lethal genes.

**Genetic diversity and divergence**

A greater number of alleles detected in the restoration population adults compared to the remnant population adults of *H. nitida* was unexpected given the reference population was the only recorded seed source for restoration. The degree of genetic divergence among the restoration and remnant population was also unexpected, and was much greater than that observed among restoration and remnant reference populations of *A. cyclops* (Millar et al, 2019a), *M. acuminata* (Millar et al. 2019b), and *B. media* (Millar et al. 2020) in the same landscape. Interestingly, the degree of genetic divergence was remarkably similar to that found between a restoration population of *H. laurina* and nearby reference remnant at the same Peniup restoration site (Fernandes 2016).
As recruitment has not been observed in the restoration population of *H. nitida*, the restoration adults we sampled are the original founder individuals, and a direct result of mating at the remnant reference site only. Increased genetic diversity in the restoration population compared to the reference population and the degree of genetic divergence among the restoration and remnant population could be explained by a number of scenarios. Such genetic patterns would be observed if (1) the seed source data has not been correctly recorded and a different population or additional seed source population was used for the restoration work; (2) the recorded remnant seed source population was much larger at the time of seed collection for the restoration work; (3) the remnant seed source population received immigrant pollen from *H. nitida* individuals located elsewhere; or (4) any combination of these scenarios. Bayesian clustering analysis clearly supports a scenario of seed being collected from a second remnant population, in addition to the one recorded and sampled here.

Our ecological genetic assessment of restoration revealed random fine-scale pollen dispersal in a restoration population of an insect-pollinated species of Proteaceae. The combination of largely equivalent mating system parameters, including the outcrossing rate, in the restoration and remnant reference seed source population, implies the attraction of animal-pollinator services within a nine-year-old restoration population in a highly fragmented landscape. While pollen immigration into the restoration population was low, potentially suggesting limited integration into the greater landscape, the random pattern of spatial pollen dispersal within the population suggest progress toward the restoration objectives through the attraction of pollinator services. Despite this, evidence for mating among related
individuals and low pollen diversity in progeny cohorts, observed for the year of sampling, suggest that small effective population size may potentially be of concern for the restoration population. Also, recruitment will be required for genetic diversity resulting from effective mating systems to be realized in subsequent generations within restoration populations. Life history traits including flowering phenology and fecundity, seed dispersal, and the extent to which outcrossing species, such as *H. nitida*, suffer negative genetic and demographic consequences of inbreeding, as well as studies of pollinator abundance and activity are all important areas of research that would contribute to further assessment of achievement of restoration aims.
Acknowledgements

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### Table 1. Characterisation of 12 microsatellite loci in adult plants from two populations of *Hakea nitida*. Details are given for locus name, primer sequences, GenBank accession number, repeat motif and the size range of observed alleles in base pairs for adult individuals. Dye label of forward primer is also given.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequence (5′-3′)</th>
<th>GenBank accession no.</th>
<th>Repeat motif</th>
<th>Allele size range</th>
<th>Dye label forward primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hn03</td>
<td>F: ACACAGTGTGGGCTGTTAGG</td>
<td>MH801951</td>
<td>(AG)&lt;sub&gt;11&lt;/sub&gt;</td>
<td>177-198</td>
<td>NED</td>
</tr>
<tr>
<td></td>
<td>R: TGACGTCTTTGCCACCATGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hn06</td>
<td>F: TTTCCCTCATGTCGCTGCC</td>
<td>MH801954</td>
<td>(AG)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>185-195</td>
<td>PET</td>
</tr>
<tr>
<td></td>
<td>R: CAACCTCTGCACCCACCAAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hn12</td>
<td>F: TCATGTATGTGGGCTGCC</td>
<td>MH801952</td>
<td>(AG)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>164-179</td>
<td>NED</td>
</tr>
<tr>
<td></td>
<td>R: CGAAAGAATCGTGTGGGCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hn13</td>
<td>F: TTTCCCTCATGTCGCTGCC</td>
<td>MH801953</td>
<td>(AAG)&lt;sub&gt;6&lt;/sub&gt;</td>
<td>131-153</td>
<td>FAM</td>
</tr>
<tr>
<td></td>
<td>R: CAACCTCTGCACCCACCAAC</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hn19</td>
<td>F: ACCTGAACCTGAGGGAGGCAG</td>
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<td>(ATCT)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>85-120</td>
<td>FAM</td>
</tr>
<tr>
<td></td>
<td>R: GGACTGCATCATGTGTTGG</td>
<td>MH801955</td>
<td></td>
<td></td>
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<tr>
<td>Hn22</td>
<td>F: TCATCTCAAACGGAGTACGC</td>
<td>MH801956</td>
<td>(AAG)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>132-154</td>
<td>FAM</td>
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<tr>
<td></td>
<td>R: AGCAATGGTTTGAGTATGGGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hn24</td>
<td>F: TAAGGAACGGAGTGGTCACG</td>
<td>MH801957</td>
<td>(AG)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>178-197</td>
<td>NED</td>
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<tr>
<td></td>
<td>R: TTCGCTCCTTCAATCGCTTAC</td>
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<td>Hn33</td>
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<td>(AGG)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>136-179</td>
<td>VIC</td>
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<td>R: CTGTTGGGTGGCAGTGTG</td>
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<tr>
<td>Hn47</td>
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<td>(AG)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>120-126</td>
<td>PET</td>
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<tr>
<td></td>
<td>R: TGATGCTCATTCTTCTTCTCC</td>
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<td>Hn52</td>
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<td></td>
<td>R: ACTCAACCTTTGTGGAGTGC</td>
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<tr>
<td>Hn55</td>
<td>F: AGTGGTCACTTGTCAGTGAC</td>
<td>MH801961</td>
<td>(AC)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>226-227</td>
<td>VIC</td>
</tr>
<tr>
<td></td>
<td>R: CTTGAACACTAGGGAGTGGTCG</td>
<td></td>
<td></td>
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<tr>
<td>Hn58</td>
<td>F: GAAGCAGACACTCATATTGTC</td>
<td>MH801962</td>
<td>(AG)&lt;sub&gt;6&lt;/sub&gt;</td>
<td>159-177</td>
<td>PET</td>
</tr>
<tr>
<td></td>
<td>R: GGTGAATGAACGTGATTGCAATC</td>
<td></td>
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</table>
Table 2. Estimates of density (plants ha\(^{-1}\)) and mating system parameters for remnant and restoration populations of *Hakea nitida* obtained using 12 microsatellite loci. Mating system parameters include the multi locus outcrossing rate (\(t_m\)), the single locus outcrossing rate (\(t_s\)), the apparent level of selfing due to biparental inbreeding (\(t_m - t_s\)), the correlation of selfing among maternal plants (\(r_s\)), the correlation of selfing among loci (\(r_{sl}\)), the multi locus correlated paternity (\(r_{pm}\)) and the effective number of pollen donors (\(N_{ep}\)). Standard deviations (population values) or standard errors (means) are in parenthesis. \(^a\)Values are significantly different to 1 (\(t_m\) and \(t_s\)) or to zero (other estimates). \(^b\)Values are significantly different to each other.

<table>
<thead>
<tr>
<th>Population</th>
<th>Density</th>
<th>(t_m)</th>
<th>(t_s)</th>
<th>(t_m - t_s)</th>
<th>(r_s)</th>
<th>(r_{sl})</th>
<th>(r_{pm})</th>
<th>(N_{ep})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peniup remnant</td>
<td>2</td>
<td>0.745(^a)</td>
<td>0.565(^a)</td>
<td>0.180(^a)</td>
<td>0.906(^a)</td>
<td>0.000</td>
<td>0.188(^{ab})</td>
<td>5.319</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.096)</td>
<td>(0.053)</td>
<td>(0.078)</td>
<td>(0.081)</td>
<td>(0.034)</td>
<td>(0.051)</td>
<td></td>
</tr>
<tr>
<td>Peniup restoration</td>
<td>23</td>
<td>0.861(^a)</td>
<td>0.675(^a)</td>
<td>0.186(^a)</td>
<td>0.901</td>
<td>0.189(^a)</td>
<td>0.519(^{ab})</td>
<td>1.927</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.050)</td>
<td>(0.070)</td>
<td>(0.048)</td>
<td>(0.040)</td>
<td>(0.030)</td>
<td>(0.114)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.803(^a)</td>
<td>0.620(^a)</td>
<td>0.183(^a)</td>
<td>0.904(^a)</td>
<td>0.095</td>
<td>0.354</td>
<td>3.623</td>
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<tr>
<td></td>
<td></td>
<td>(0.058)</td>
<td>(0.055)</td>
<td>(0.003)</td>
<td>(0.003)</td>
<td>(0.095)</td>
<td>(0.166)</td>
<td>(1.696)</td>
</tr>
</tbody>
</table>
Table 3. The number of individuals sampled (No), mean number of individuals genotyped averaged over all loci (N) and genetic diversity parameters for 12 microsatellite loci in adult and progeny cohorts of remnant and restoration populations of *Hakea nitida* and the means of the two populations for each cohort. Genetic diversity parameters include the proportion of polymorphic loci (Po), the mean number of alleles per locus (Na), the mean rarified number of alleles (Nar), the number of private alleles (P), the number of private alleles after rarefaction (Pr), the effective number of alleles (Ne), expected (H_e) and observed (H_o) heterozygosity, and the Fixation index (F). Standard errors are in parenthesis. 

<table>
<thead>
<tr>
<th>Sample site or Region</th>
<th>No</th>
<th>N</th>
<th>Na</th>
<th>Nar</th>
<th>Po</th>
<th>Pr</th>
<th>Ne</th>
<th>P</th>
<th>H_e</th>
<th>H_o</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adults</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peniup remnant</td>
<td>20</td>
<td>18.500</td>
<td>4.583 (0.544)</td>
<td>4.388b (0.598)</td>
<td>18</td>
<td>1.277b (0.266)</td>
<td>2.450 (0.374)</td>
<td>91.67 (0.076)</td>
<td>0.481 (0.092)</td>
<td>0.449 (0.103)</td>
<td>0.103 (0.093)</td>
</tr>
<tr>
<td>Peniup restoration</td>
<td>172</td>
<td>142.750</td>
<td>11.833 (8.389)</td>
<td>6.993b (0.977)</td>
<td>40</td>
<td>3.883b (1.303)</td>
<td>4.326 (0.794)</td>
<td>91.67 (0.089)</td>
<td>0.625 (0.081)</td>
<td>0.494 (0.100)</td>
<td>0.272a (0.100)</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>96</td>
<td>80.625</td>
<td>8.208 (13.591)</td>
<td>5.691 (1.245)</td>
<td>29</td>
<td>2.850 (1.303)</td>
<td>3.388 (0.155)</td>
<td>91.67 (0.059)</td>
<td>0.553 (0.060)</td>
<td>0.471 (0.072)</td>
<td>0.188a (0.072)</td>
</tr>
<tr>
<td><strong>Progeny</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Peniup remnant</td>
<td>107</td>
<td>55.917</td>
<td>6.833 (8.124)</td>
<td>5.170 (0.851)</td>
<td>45</td>
<td>5.170 (0.628)</td>
<td>3.070 (0.533)</td>
<td>100 (0.064)</td>
<td>0.570 (0.071)</td>
<td>0.327 (0.077)</td>
<td>0.484a (0.077)</td>
</tr>
<tr>
<td>Peniup restoration</td>
<td>752</td>
<td>585.750</td>
<td>12.083 (28.296)</td>
<td>6.121 (1.510)</td>
<td>43</td>
<td>6.121 (0.655)</td>
<td>3.379 (0.558)</td>
<td>100 (0.052)</td>
<td>0.618 (0.067)</td>
<td>0.440 (0.076)</td>
<td>0.321a (0.076)</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>429.5</td>
<td>320.833</td>
<td>9.458 (57.019)</td>
<td>5.646 (1.009)</td>
<td>44</td>
<td>2.371 (0.476)</td>
<td>3.225 (0.379)</td>
<td>100 (0.041)</td>
<td>0.594 (0.049)</td>
<td>0.384 (0.056)</td>
<td>0.402a (0.056)</td>
</tr>
</tbody>
</table>
Figure Captions

Figure 1. Map of the study area showing the remnant reference and restoration study population of *Hakea nitida*. All trees present and sampled for leaf material at the restoration study population are indicated (white circles) and maternal trees sampled for seed are indicated (red circles). The darker landscape is remnant vegetation. The lighter landscape is typically agricultural crop or young revegetation. The inset map shows records of *Hakea nitida* (red circles) and indicates the study region (black rectangle).

Figure 2. Comparison of the frequency distributions of the proportions of realised pollen dispersal distances (dark bars) and the frequency distributions of the proportions of pair-wise distances between all plants (light bars), using paternity assignment for the study area of *Hakea nitida* restoration. There was no statistically significant difference between the two dispersal frequency patterns (*p* = 0.9829).

Figure 3. Assignment of individuals to one of two genetic clusters identified via Bayesian analysis of multilocus nuclear microsatellite genotype data for adult individuals of both the reference remnant population and restoration population of *Hakea nitida*. Each individual is represented as a vertical line partitioned into K-coloured segments (red or green) whose length is proportional to the individual coefficients of membership (Q) in the two clusters.