Department of Biodiversity, Conservation and Attractions

GOVERNMENT OF WESTERN AUSTRALIA

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Is restoration working? An ecological genetic assessment

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VIRTUS IMPAVIDA

Project Background

- 3 year project
- Assess the success of restoration
- Ecological and genetic viability
- In the Fitzgerald River–Stirling Range region

- Moves measures of restoration success beyond that of population establishment and survival
- Incorporates the evolutionary processes that provide
  - Short-term resilience
  - Long-term persistence and
  - Functional integration of restored populations into broader landscapes

Project Background

Hypothesis: ecologically and genetically viable restoration populations will mimic natural vegetation

More likely to persist in the long term and contribute to effective ecosystem function through integration into the broader landscape

Is restoration working? Questions

- Is available genetic diversity being captured?
- Is appropriate genetic diversity being captured?
- Are mating systems functional?

Compare
- genetic diversity
- genetic divergence
- mating systems

among restoration populations and remnant populations

- What are the patterns of pollen dispersal within restored populations?
- Is there pollen immigration into restoration populations from outside?

- Direct paternity analysis in restoration populations

Sites

Up to four restoration sites (depending on species) established with

- differing seed and seedling establishment regimes
- for differing lengths of time (5-16 years)
- and with different degrees of consideration of landscape age and fertility

Chereninup Creek Reserve 2003

Chingarrup Sanctuary 2005

Peniup Creek Reserve 2008

Monjebup North Nature Reserve 2012

Species

- Five species
- Different genera
- Foundation or framework species

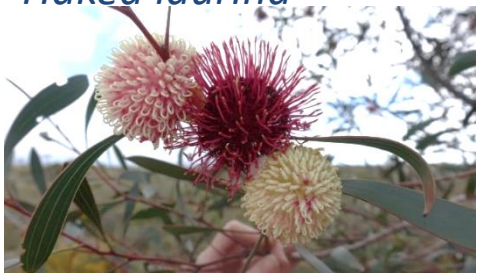
- Used over the broader landscape
- All animal pollinated

Acacia cyclops

Melaleuca acuminata

Banksia media

Hakea laurina



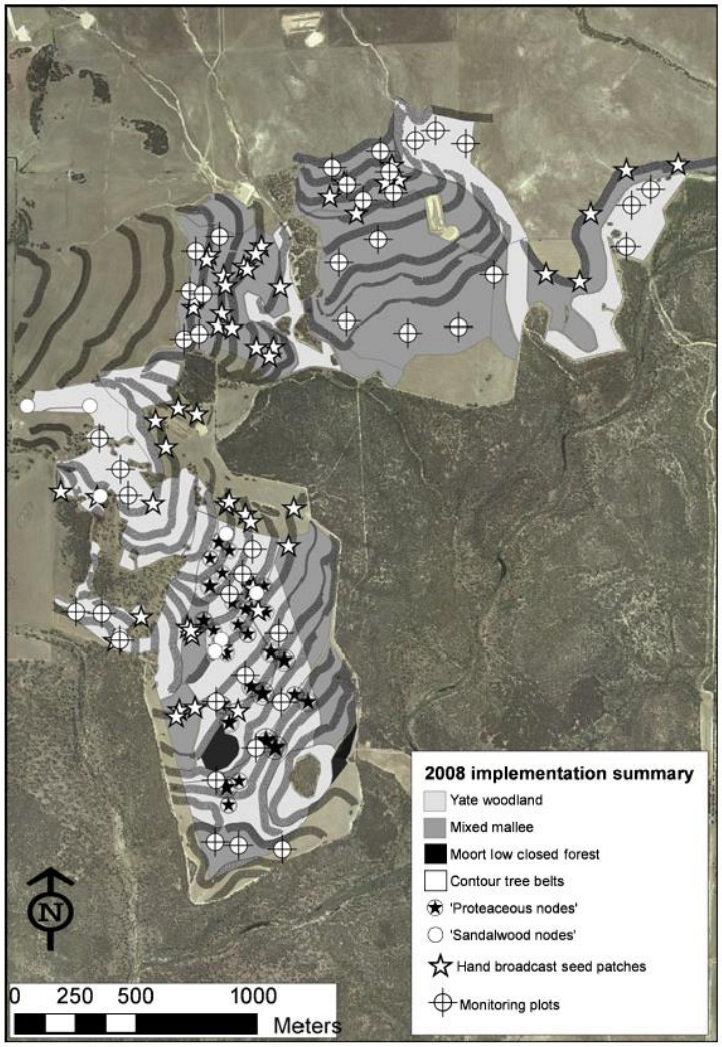
Hakea nitida

Sites and Species

Species	Pollinator	Chereninup	Chingarrup	Peniup	Monjebup	Method	Spatial
Acacia cyclops	Insects	✓	✓	✓	Too young	Direct seeding	Tens of meters
Melaleuca acuminata	Insects	✓	✓	✓	✓	Direct seeding	Dense
Banksia media	Birds Honey possums Insects			✓	✓	Hand	Peniup - nodes Monjebup – grid
Hakea nitida	Insects Birds?			✓	Too young	Hand	Peniup – nodes
Hakea laurina	Insects Birds	✓		✓	Too young	Hand	Peniup - nodes Chereninup - tens of meters

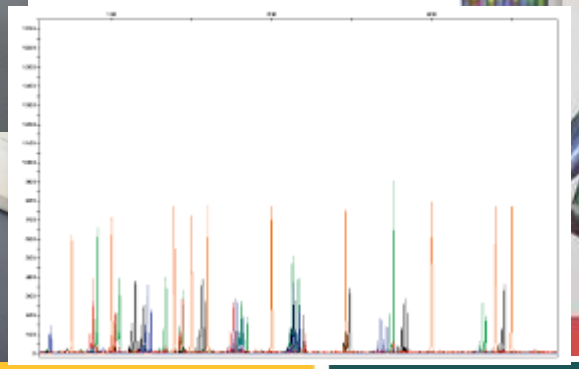
Proteaceous species at Peniup

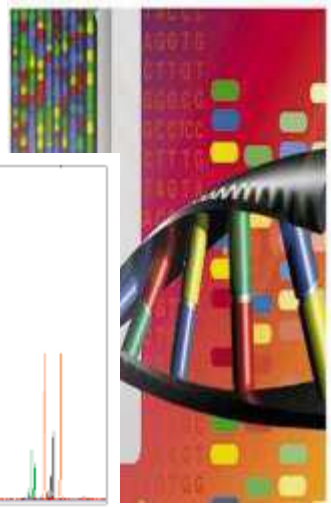
- Nodes of 10 to 50 individuals
- Mimic patchy distribution in remnant vegetation
- Maximise the impact of animal pollinators
- Contrast with
  - Monjebup - for *B. media* grid every 30 m
  - Chereninup - for *H. laurina* widely spaced



Approach

- Sample leaf and seed
- Germinate seed
- Extract DNA from leaf and seed
- Genotype all individuals with 10-12 microsatellite markers
- Assess genetic diversity and genetic divergence
- Assess mating systems
- Assess pollen dispersal via direct paternity analysis of seed - *Banksia media* and *Hakea nitida*

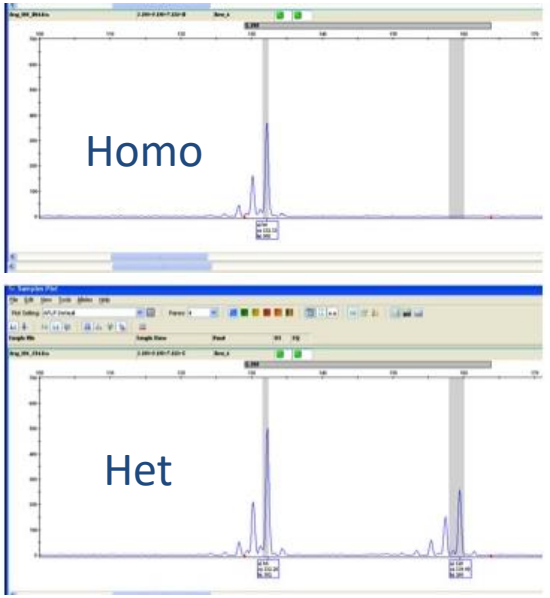




Genetics

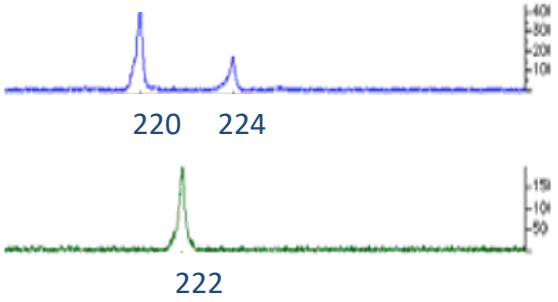
2 stranded DNA

2 alleles per locus



Within an individual 2 alleles can be

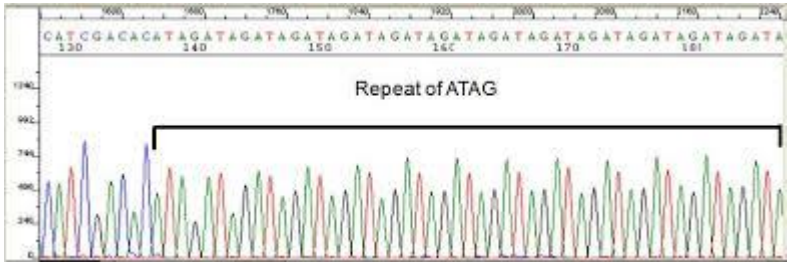
- the same (homozygous)
- or different (heterozygous)



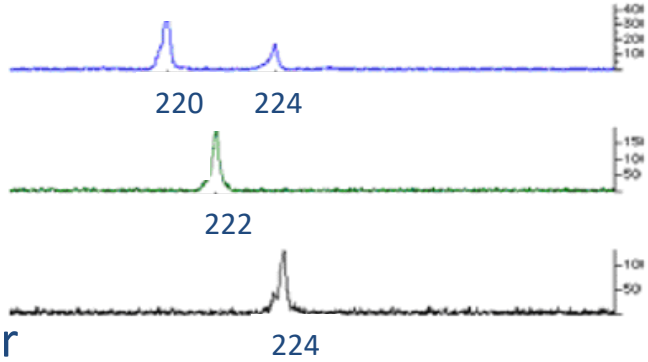
- The alleles present within individuals may differ
- The frequency distributions of alleles represent population genetic diversity
- Between a population allele frequencies can also differ – genetic divergence

Genetics – microsatellite genotyping

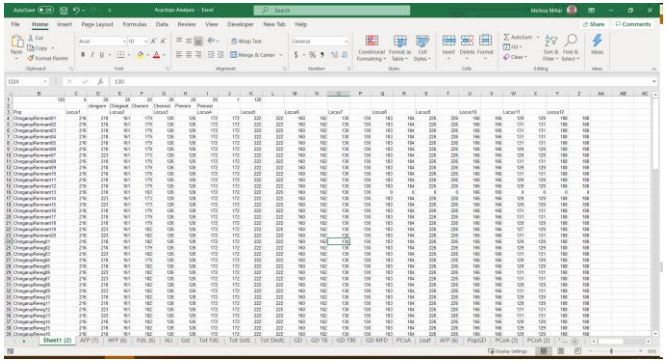
- A genetic marker
- A small sequence of repetitive DNA
- Typically 2 -5 base pairs
- Repeated 5-50 times



- Highly variable in repeat number and length due to slippage in DNA replication



- Design and optimise 12 microsatellite markers for each species
- Genotype all individuals at 12 loci – multilocus genotypes
- Statistically powerful data set



Is restoration working? I Genetic Diversity

Is available genetic diversity being captured?

Genetic diversity is important

- Resilience to disturbance
- Evolutionary potential for adaptation
- Short-term and long-term persistence
- Maintains mating systems and negate negative effects of inbreeding depression
- Related to the size and diversity of the seed source population/s
- Pollinator diversity and effectiveness

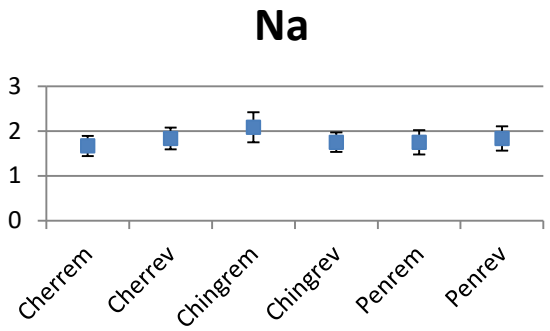
Measures of genetic diversity

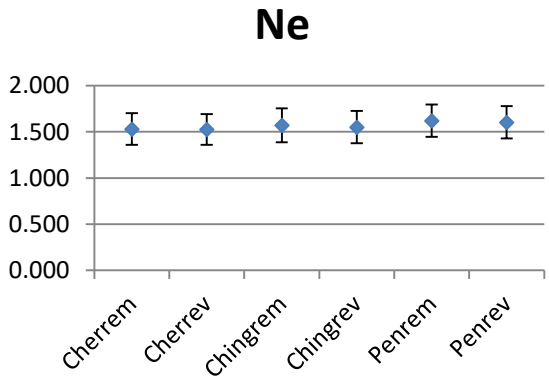
- Na - number of alleles per locus
- Ne - effective number of alleles per locus (related to allele frequency)
- Nar – rarefied number of alleles per locus corrected for differences in sample size
- He and Ho – expected and observed heterozygosity

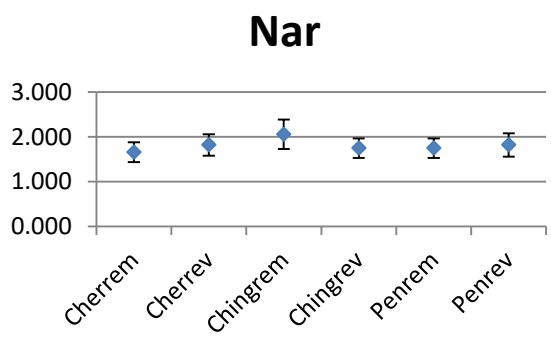
Assess for differences among sites and treatments (restoration vs remnant populations) using Analysis of Variance ANOVA

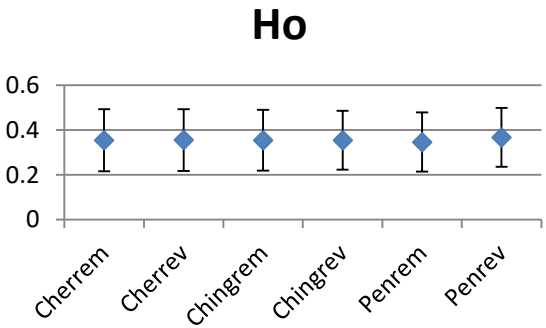
Genetic Diversity - *Acacia cyclops*

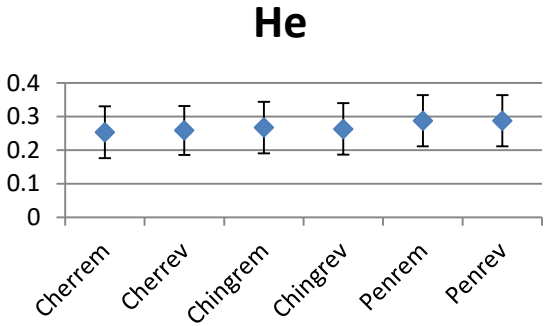
- Similar levels of diversity in restored populations and remnants
- Seed collections have captured available diversity
- Low diversity
  - Disturbed small remnants







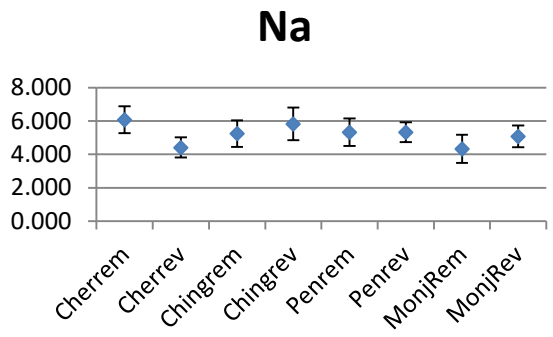


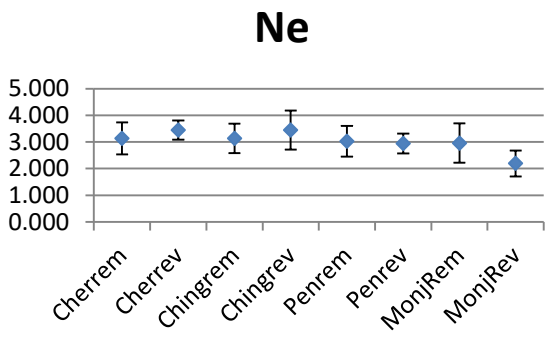


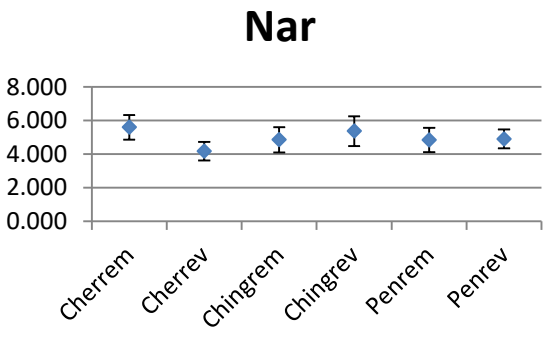
No significant differences among sites or treatments (remnant/restoration) ANOVA

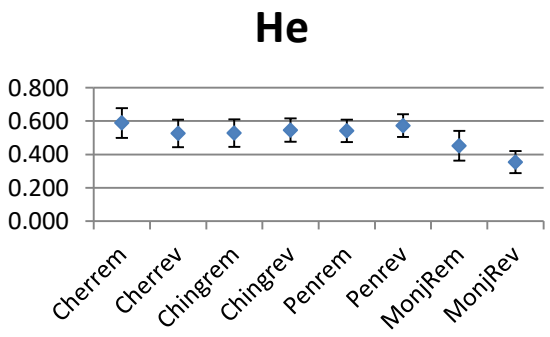
Genetic diversity – *Melaleuca acuminata*

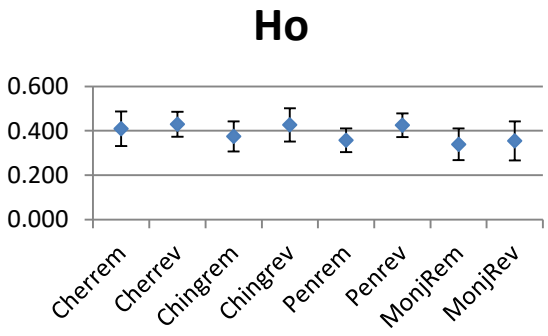
- Similar levels of diversity in restored populations and remnants
- Seed collections have captured available genetic diversity







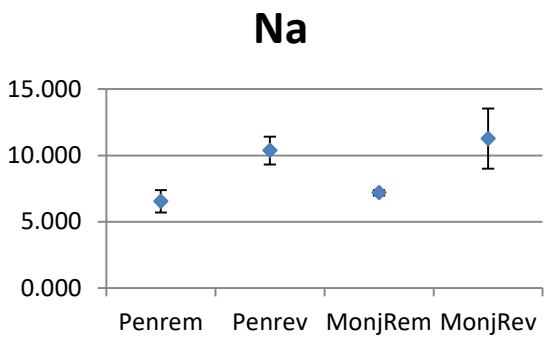


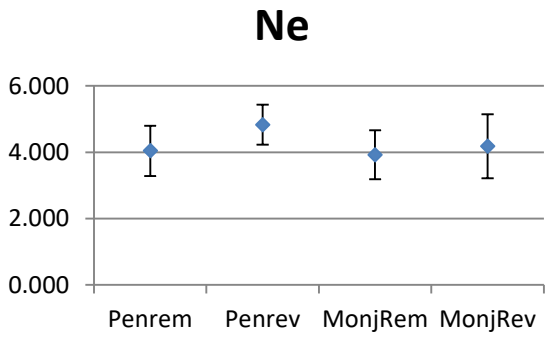


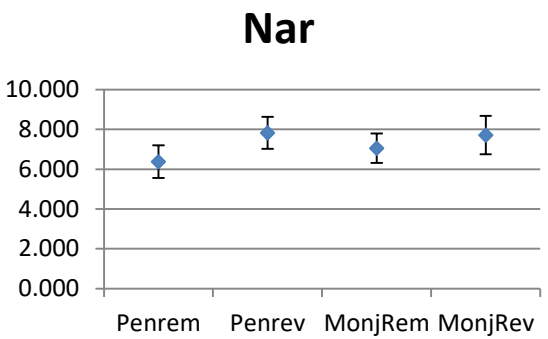
No significant differences among sites or treatments (remnant/restoration) ANOVA

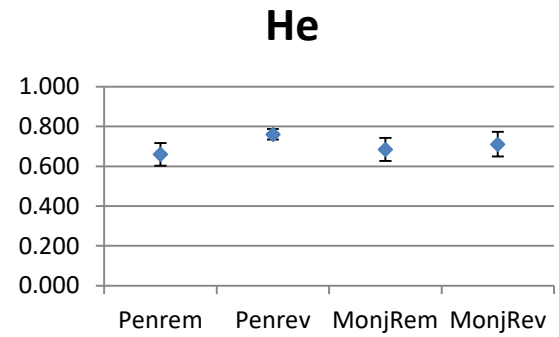
Genetic diversity – *Banksia media*

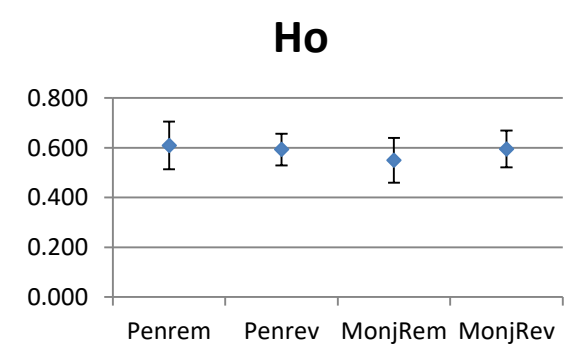
- More alleles and effective alleles per locus in restoration compared to remnants – but not after rarefaction
- Similar levels of diversity in restored populations and remnants
- Seed collections have captured available genetic diversity







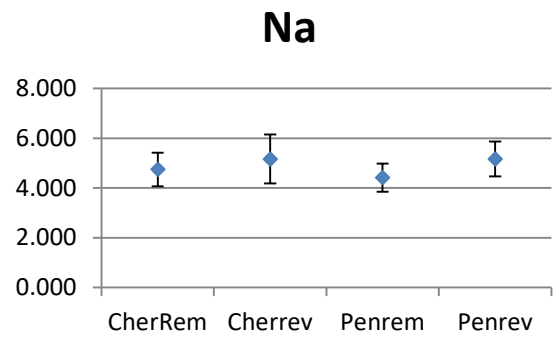


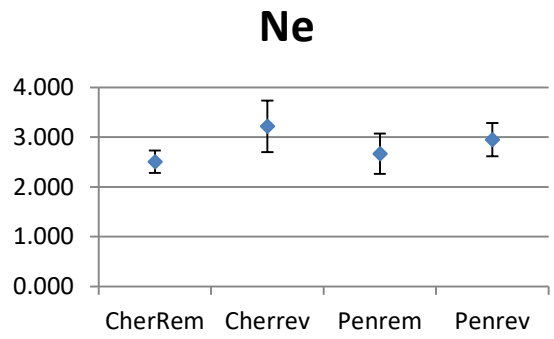


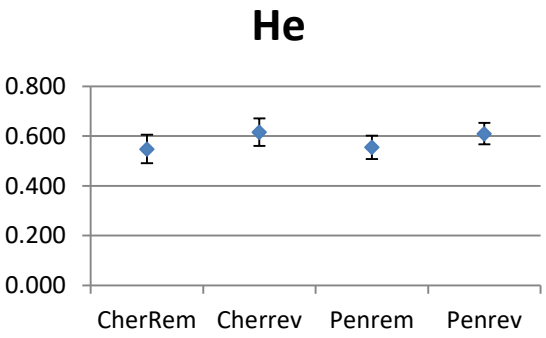
No significant differences among sites ANOVA

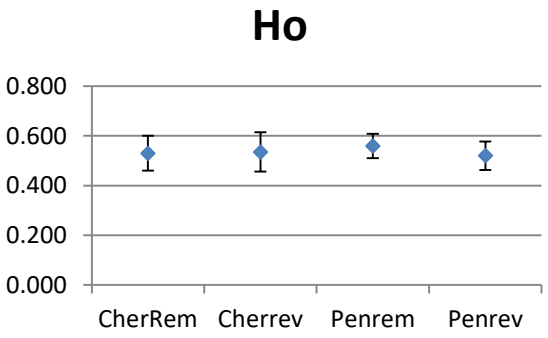
Genetic diversity – *Hakea laurina*

- Similar levels of diversity in restored populations and remnants
- Seed collections have captured available genetic diversity





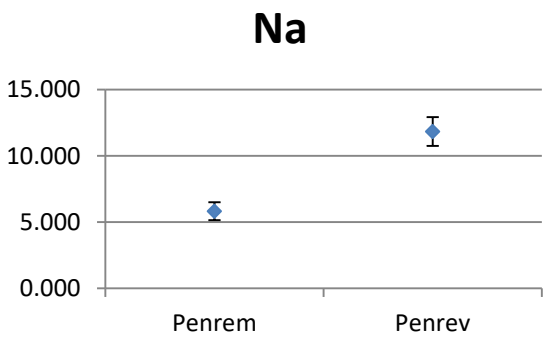


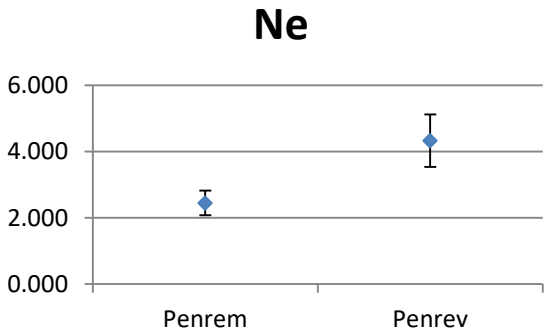


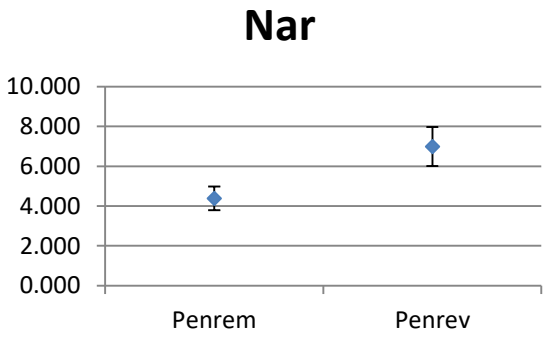
No significant differences among sites or treatments (remnant/restoration) ANOVA

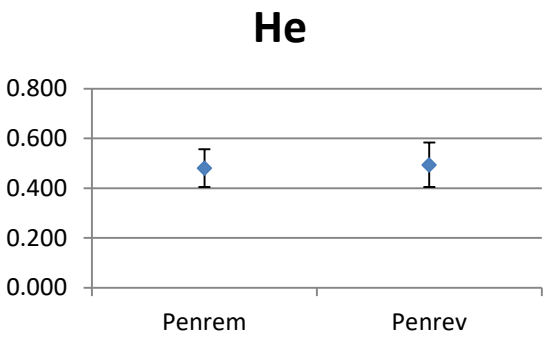
Genetic diversity – *Hakea nitida*

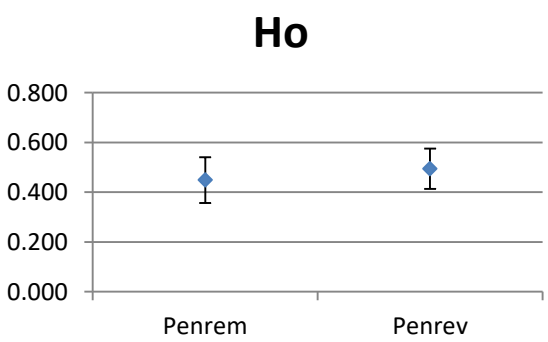
- Peniup only – one recorded seed source
- More alleles per locus, effective number of alleles in restoration even after rarefaction
  - Additional seed sources?
  - Recorded source population was bigger at time of seed collection?











Is restoration working I Genetic divergence

Is genetic diversity of local provenance being captured?

Sourcing seed of local provenance is important

- A precautionary approach
- Well suited to local environmental conditions
- Avoids mixing of potential cryptic divergent lineages
- Avoids potential outbreeding depression
- Avoids other potential negative ecological interactions eg invasiveness

Measure of genetic divergence

D_{ST} - Variance in alleles found among populations

Genetic divergence – *Acacia cyclops*

- Little divergence between restoration and remnants
- Suggests seed collections were of local provenance

D_{ST}	Chingrem	Chingrest	Cherrem	Cherrest	Penrem	Penrest
Chingrem	0.000					
Chingrest	**0.009**	0.000				
Cherrem	0.035	0.023	0.000			
Cherrest	0.088	0.067	**0.040**	0.000		
Penrem	0.031	0.031	0.024	0.053	0.000	
Penrest	0.042	0.018	0.027	0.021	**0.030**	0.000

Genetic divergence – *Melaleuca acuminata*

- Little divergence among restoration and remnants
- Suggests seed collections of local provenance

D_{ST}	ChingRem	ChingRest	CherRem	CheRest	PenRem	PenRest	Mrem	Mrest
ChingRem	0.000							
ChingRest	**0.016**	0.000						
CherRem	0.064	0.036	0.000					
CherRest	0.079	0.070	**0.067**	0.000				
PenRem	0.027	0.031	0.066	0.059	0.000			
PenRest	0.043	0.044	0.041	0.061	**0.023**	0.000		
Mrem	0.029	0.034	0.059	0.085	0.047	0.083	0.000	
Mrest	0.034	0.025	0.058	0.067	0.027	0.000	**0.055**	0.000

Genetic divergence - Proteaceae

- Divergence at Peniup for *Banksia media, Hakea laurina and Hakea nitida*
- Divergence at Chereninup for *Hakea laurina*
  - Wider seed sourcing?

Banksia media

D_{ST}	PenReveg	PenRem	MonjReveg	MonjRem
PenReveg	0.000			
PenRem	**0.164**	0.000		
MonjReveg	0.194	0.215	0.000	
MonjRem	0.192	0.273	**0.094**	0.000

Hakea laurina

D_{ST}	Cherrem	Cherrest	Penrem	Penrest
Cherrem	0.000			
Cherrest	**0.238**	0.000		
Penrem	0.084	0.215	0.000	
Penrest	0.085	0.228	**0.112**	0.000

Hakea nitida

D_{ST}	PenReveg	PenRem
PenReveg	0.000	
PenRem	**0.239**	0.000

Genetic divergence – *Banksia media*

- No seed collection records
- High divergence among restoration and potential seed source at Peniup ~ 1.5km
- But also high divergence among remnants ~25km apart
  - Is high divergence among populations of *B. media* typical?
  - Were seed sources in fact quite local?

- Records of seed sources would be informative

Banksia media

D_{ST}	PenReveg	PenRem	MonjReveg	MonjRem
PenReveg	0.000			
PenRem	**0.164**	0.000		
MonjReveg	0.194	0.215	0.000	
MonjRem	0.192	0.273	**0.094**	0.000

Genetic divergence – *Hakea laurina*

- Again no seed collection records
- But high divergence among restorations and potential seed sources -adjacent to ~8km
- Divergence among remnant populations is low ~15km
  - Suggests wider seed sourcing ie over much greater than 15 km

Hakea laurina

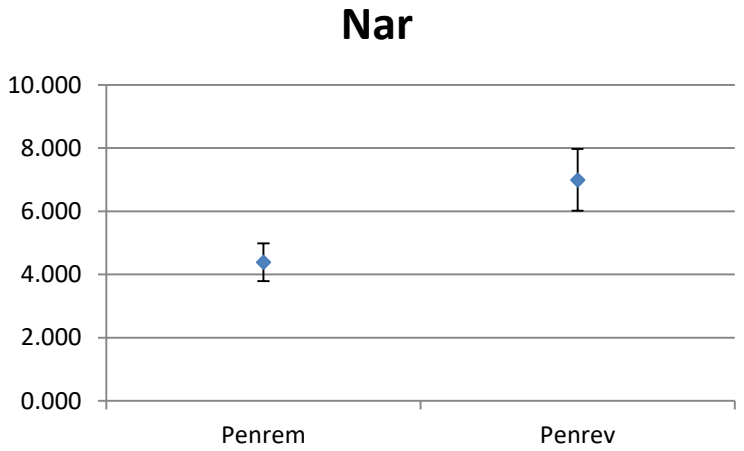
D_{ST}	Cherrem	Cherrest	Penrem	Penrest
Cherrem	0.000			
Cherrest	**0.238**	0.000		
Penrem	0.084	0.215	0.000	
Penrest	0.085	0.228	**0.112**	0.000

Genetic divergence – *Hakea nitida*

- One recorded seed source ~2km
- But high divergence
  - Suggests additional/alternative seed sourcing to nearby remnants
  - Supported by greater diversity in restoration

Hakea nitida

D_{ST}	PenReveg	PenRem
PenReveg	0.000	
PenRem	**0.239**	0.000



Is restoration working? I

- Is similar genetic diversity being captured?
  - Yes ☺ genetic diversity available in remnant populations has been captured

- Was seed sourced from local provenance?
  - Yes for fecund *Acacia* and *Melaleuca* ☺
  - Some divergence for Proteaceous species
  - Additional or alternative non local seed sources?

- Generally good outcomes consistent with restoration aims
  - When recruitment occurs not all seed will contribute
  - Will genetic diversity be maintained in future generations?
  - Depends on the maintenance of the mating system and patterns of pollen dispersal – and of course – recruitment!

Is restoration working? II

- Are mating systems functional?
- Is pollination resulting in comparable outcrossing rates?
- Any relation to population fitness measures?

- What are the patterns of pollen dispersal within restoration populations?
- Is there pollen immigration into restoration populations from outside?
  - Direct paternity analysis in restoration populations

- Are animal pollinators present and effective?

Mating Systems

- Mixed mating model
  - self-fertilised (selfed)
  - or cross-fertilised (outcrossed)
- Plant mating systems are important in
  - Avoiding effects of biparental inbreeding - mating among relatives
  - Maintaining genetic diversity
- Maintaining long-term adaptive potential
- Animal pollinated species - largely outcrossed
- Outcrossing suggests
  - presence,
  - pollen movement and
  - effective pollination by animal pollinators

Mating Systems - approach

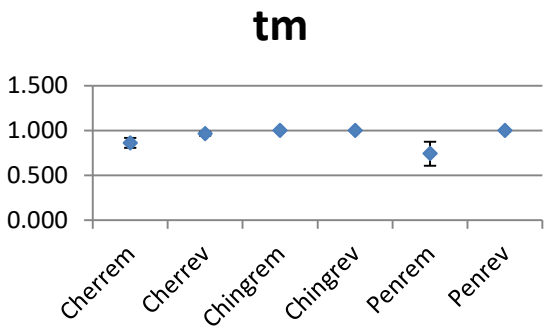
- Genotype progeny arrays (~15 seed) from a subset of mothers (10)
- We can calculate mating system parameters
  - t_m - multilocus outcrossing rate (0-1)
  - t_m-t_s– mating among relatives
  - rpm - multilocus correlated paternity - how similar fathers are per maternal plant
  - Ne = 1/rpm – effective number of pollen donors per maternal plant/pollen diversity

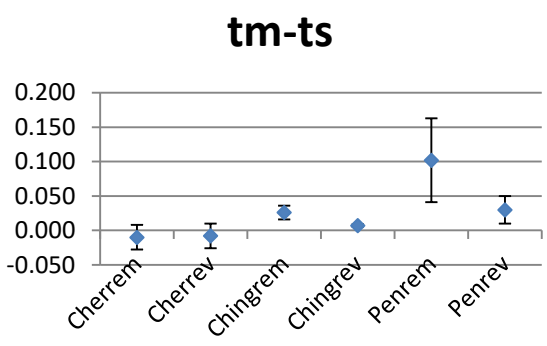
Population fitness - approach

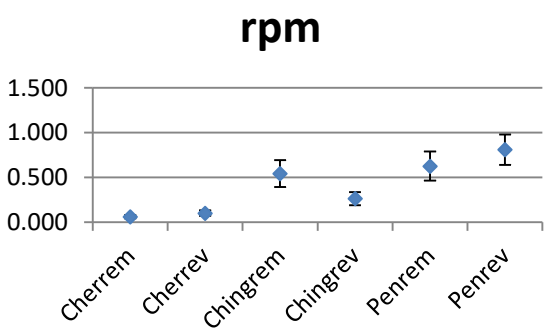
- Seed weight is a good predictor of
  - seed germination
  - seedling growth and
  - seedling survival
- Reproductive measures as proxy measures of population fitness
  - seed volume
  - number of seed per capsule
  - % viable seed
- Conduct regression analysis to determine if seed parameters are correlated to mating system parameters

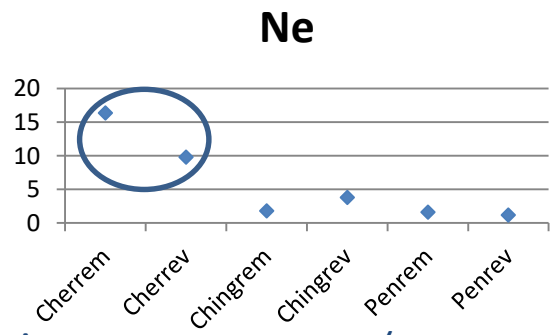
Mating Systems – *Acacia cyclops*

- Mixed mating, high outcrossing
- Pollinator services appear to be effective
- Chereninup – high number of effective pollen donors
  - right next to the dam, a water source for insects? feral bees?





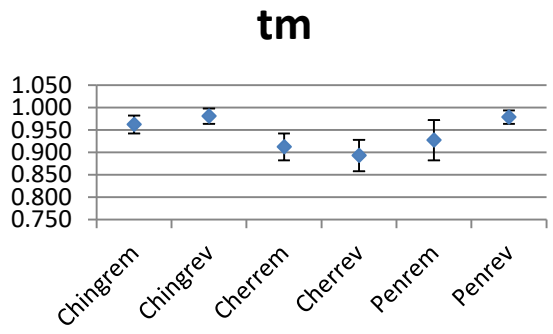


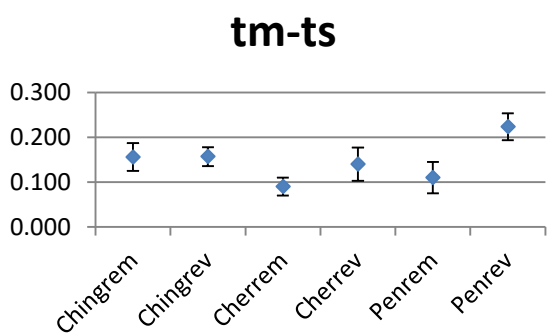


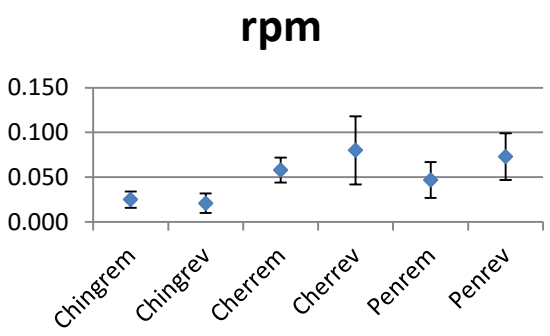
No significant differences among sites or treatments (remnant/restoration) ANOVA

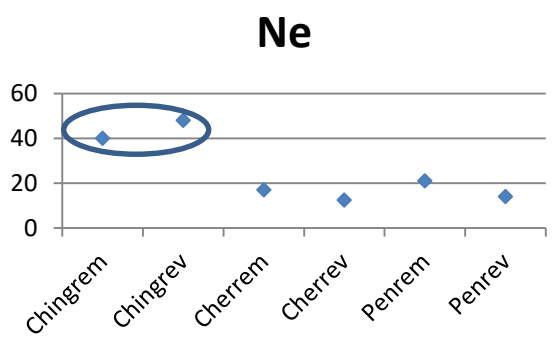
Mating Systems – *Melaleuca acuminata*

- Mixed mating, high outcrossing
- Pollinator services appear to be effective
- Many pollen donors – very high at Chingarrup
  - Increased pollinator abundance? Or pollinator richness?





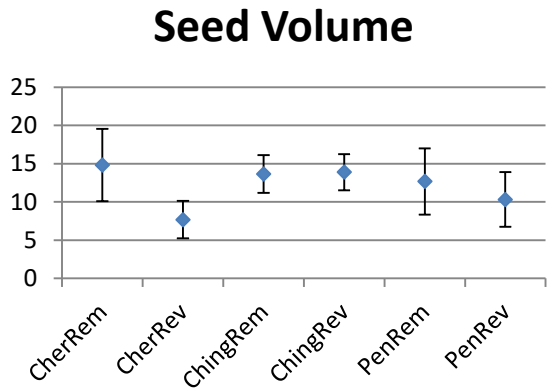


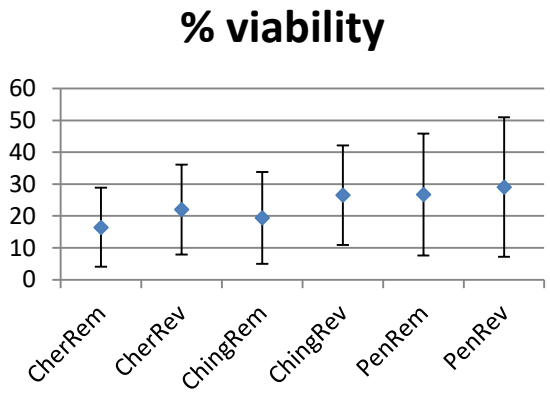


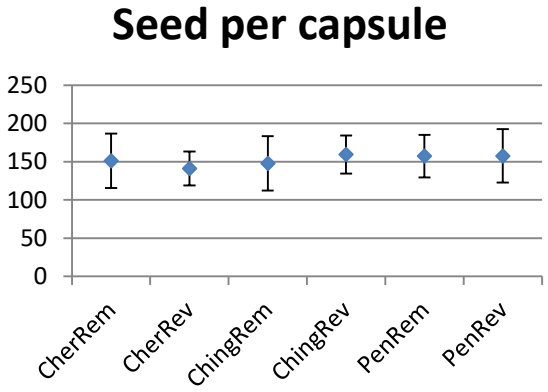
No significant differences among sites or treatments (remnant/restoration) ANOVA

Population fitness – *Melaleuca acuminata*

- No significant differences among sites or treatments (remnant/restoration) via ANOVA
- Low seed viability – but lots of it

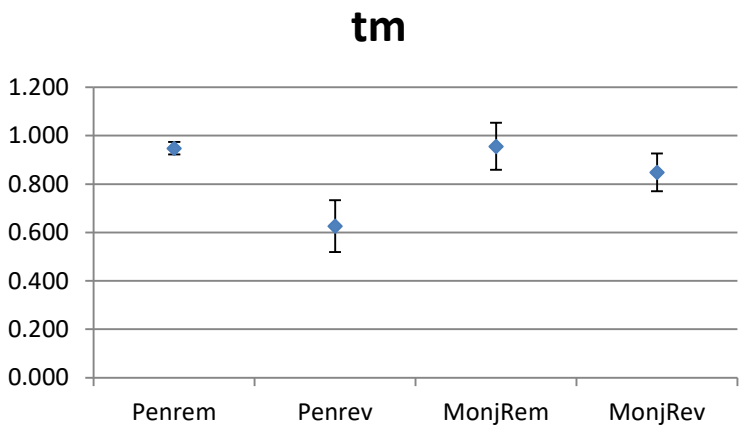






Mating Systems – *Banksia media*

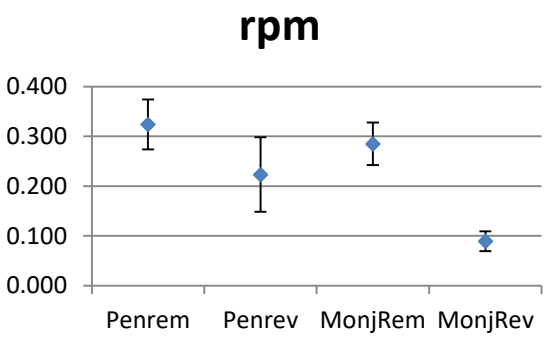
- Mixed mating, high outcrossing
- Significantly lower outcrossing at Peniup restoration than its remnant reference
  - Plants were relatively small and flowering limited
  - Fewer animal pollinators?
  - Less chance for outcrossing events?

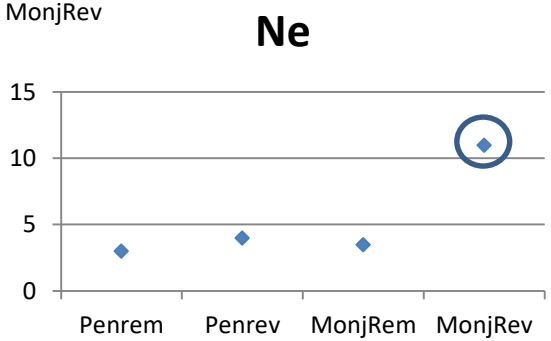


No significant differences among sites ANOVA

Mating Systems – *Banksia media*

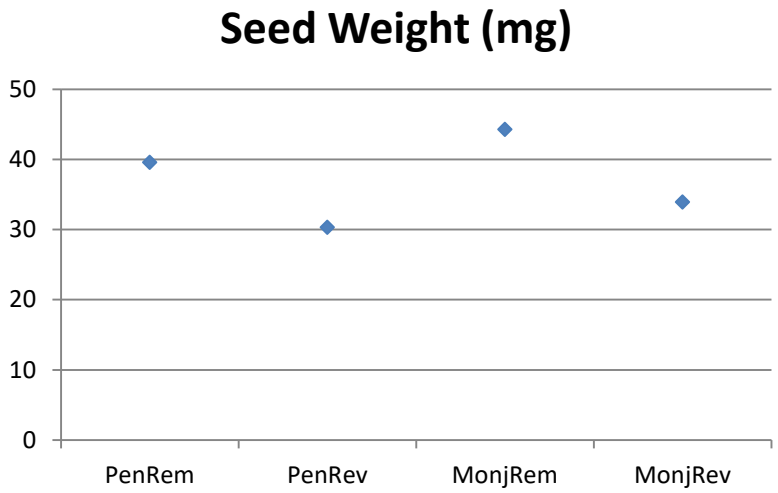
- Relatively low number of effective pollen donors at all populations
  - Are birds and honey possums less active or less abundant than insects are?
- Monjebup North - far greatest number of pollen donors
  - more open vegetation?
  - spatial arrangement of plants?





Mating system and population fitness – *Banksia media*

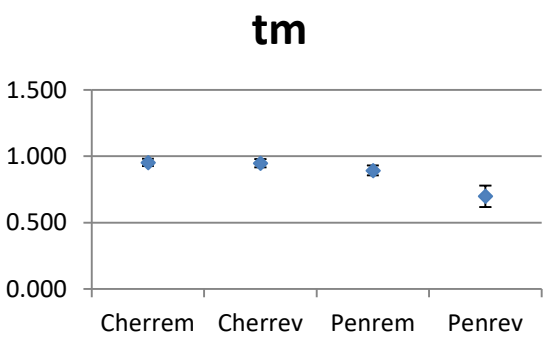
- Seed weight significantly greater in remnants
  - Related to age of trees in remnants vs restorations?
- But seed weight also significantly greater at Monjebup (4yo) compared to Peniup (8yo)
- Not correlated to outcrossing rate or number of pollen donors
  - Related to resource limitation at Peniup?

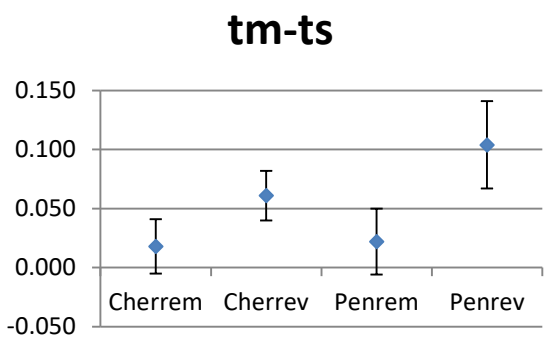


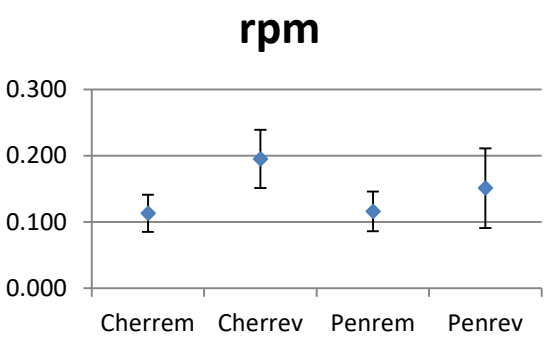
Mating Systems – *Hakea laurina*

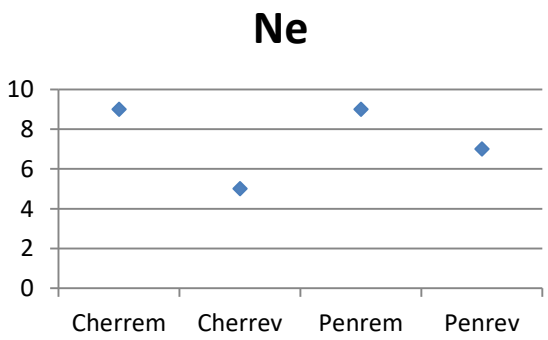
Peniup restoration - Significantly lower outcrossing and greater mating among relatives

- Located in the same area of restoration
- Some limitation to animal pollinators?









Insect floral visitors – *Hakea laurina*

- Ten trees from each population, swept twice a day
- 1111 specimens identified to species, family or genus
- ANOVA to assess abundance and richness
- 158 insect species/families
- Greater insect abundance at remnants compared to restorations
- Greater insect abundance at Peniup than Chereninup
- Feral honeybees the dominant visitors with no differences among sites or treatments
- Why lower outcrossing at Peniup? – vertebrate pollinator dependent but limited?

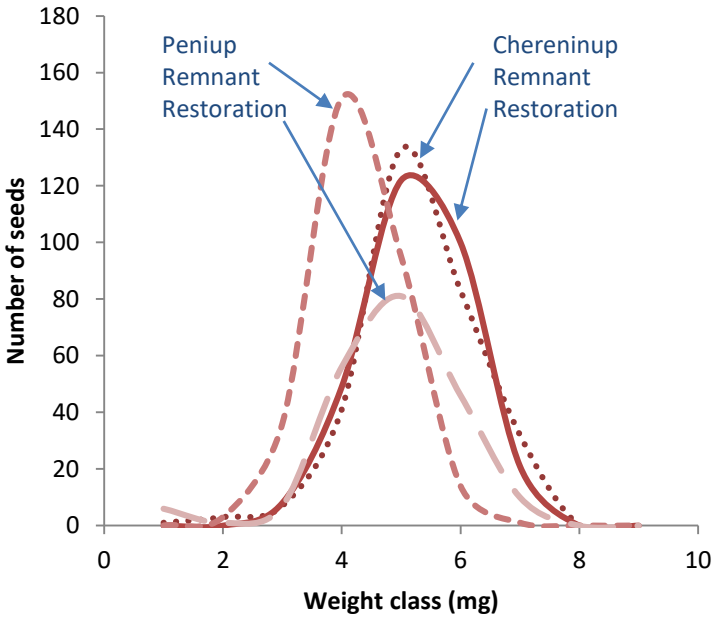
Mating Systems and population fitness – *Hakea laurina*

Peniup seed significantly lighter than at Chereninup

- resource limitations at Peniup?

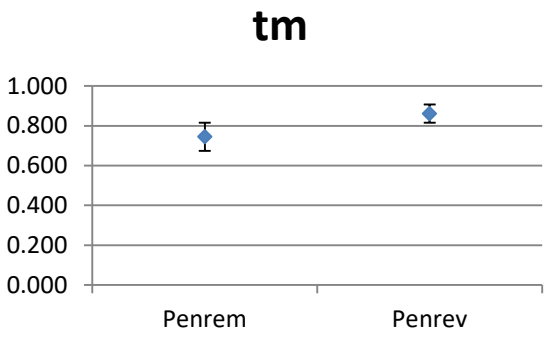
Lower seed weight and outcrossing rate were correlated at the Peniup restoration

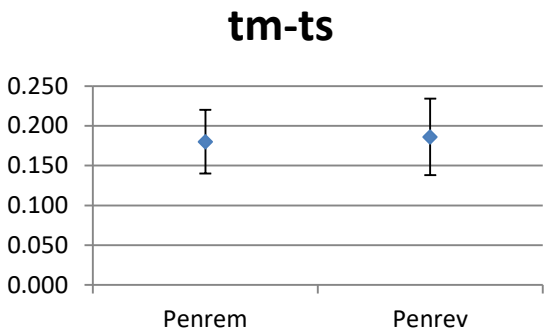
- may be of concern at Peniup for this species



Mating Systems – *Hakea nitida*

- Mixed mating, high outcrossing
- Restoration comparable to remnant

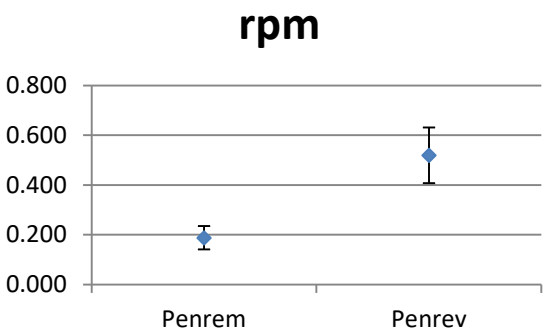


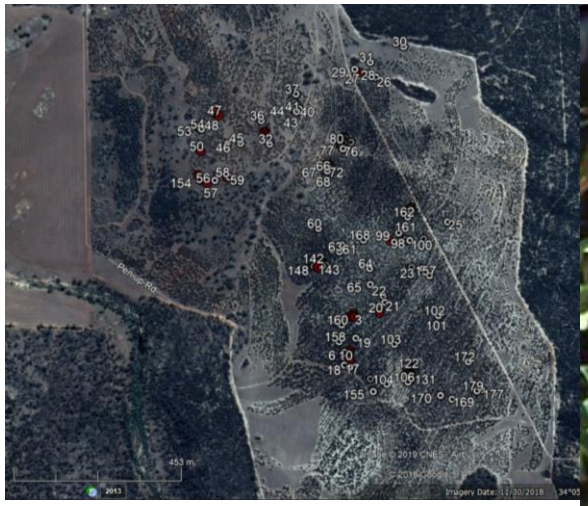


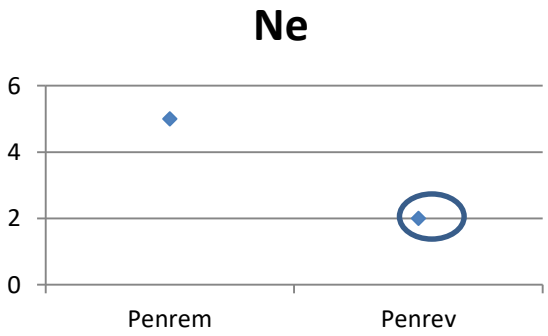
No ANOVAs as only two populations

Mating Systems – *Hakea nitida*

- Peniup restoration – again very few pollen donors







No ANOVAs as only two populations

Pollen Dispersal

- Is gene flow via pollen dispersal effective within restored populations?
  - Related to the mating system
  - Pollinator assemblage
  - Pollinator abundance and
  - Pollinator behaviour

- Is there pollen immigration into restorations from plants located outside?
  - Gene flow = connectivity at the greater landscape scale
  - Prevents inbreeding depression and genetic drift by maintaining
    - genetic diversity
    - mating systems and
    - population fitness

- Direct paternity analysis in restoration population

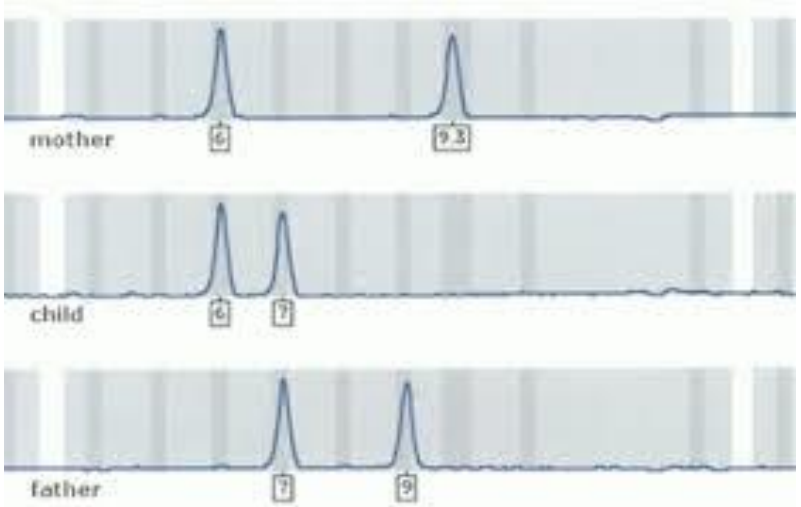
Paternity Analysis

- Genotype all plants within a given focal area
- All mature plants are potential pollen donors

- Genotype progeny from a subset of mother plants

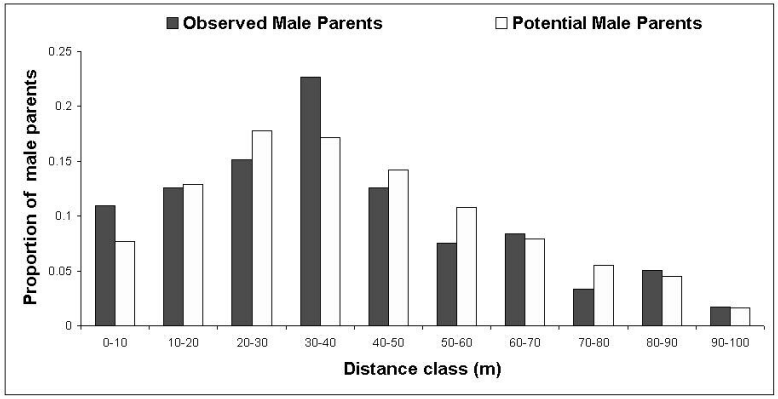
- We can then deduce the most likely father from multilocus genotypes
  - log likelihood statistics gives confidence levels for potential fathers

- The father could also be the mother (selfed)

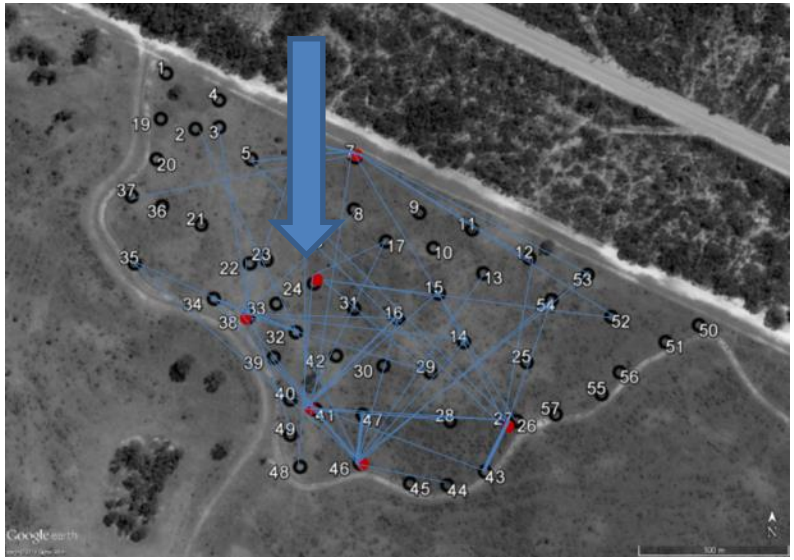


Pollen dispersal

- Map every plant in the focal area
- Calculate potential pollen dispersal in different distance classes
- Compare this to realised pollen dispersal in different distance classes



- We can map pollen dispersal events



- Pollen immigration - no most likely father inside the focal area

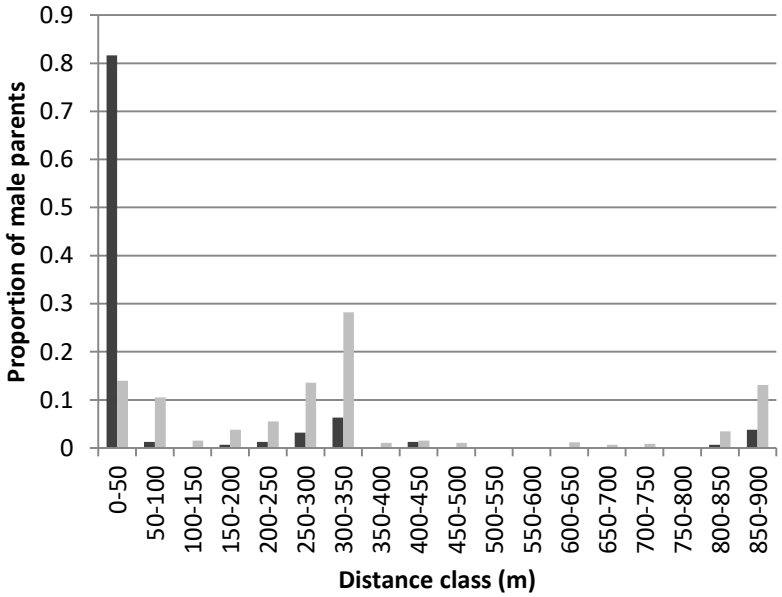
Pollen dispersal – *Banksia media*

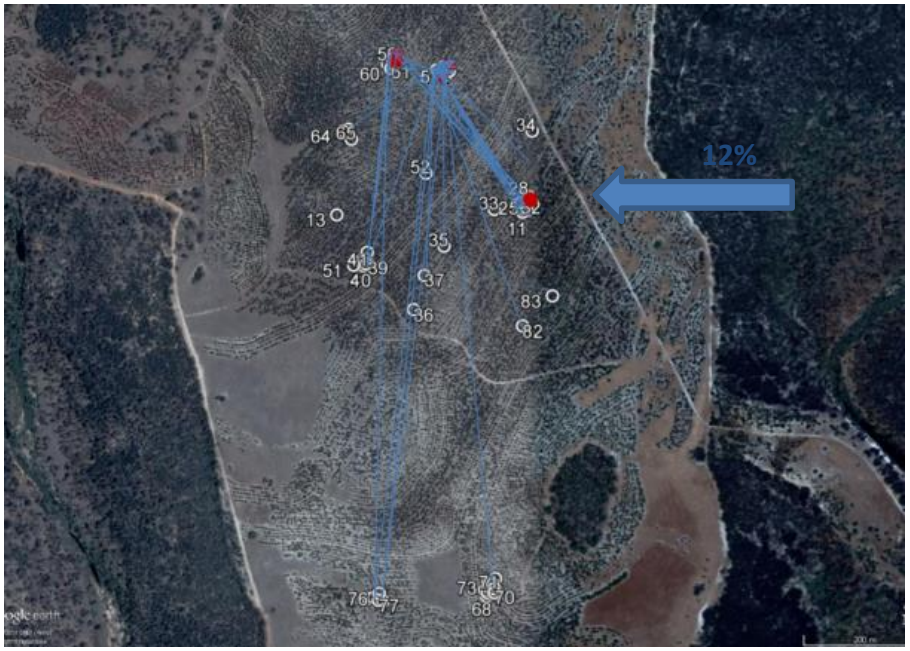
Peniup

- Nodes

- Vast majority of pollen dispersal is in the shortest distance class ie 0-50m or within nodes
- Few medium and long distance dispersal events

- Pollen immigration from outside the 41ha = 12%





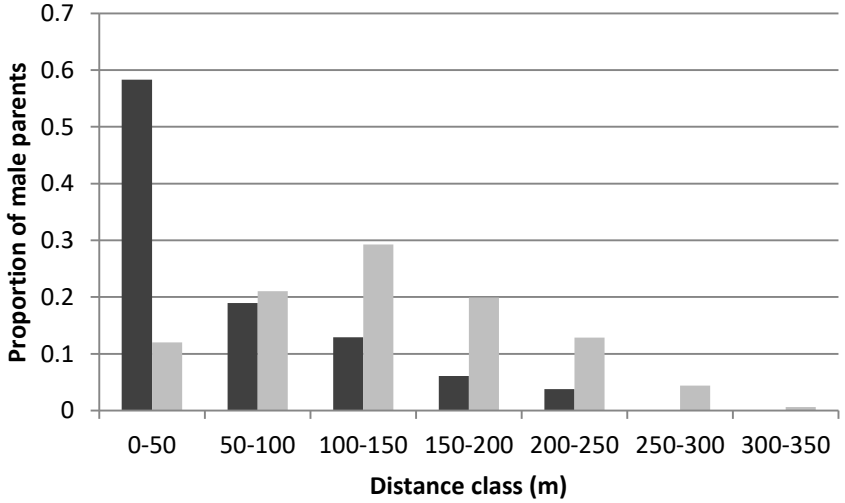
Pollen dispersal – *Banksia media*

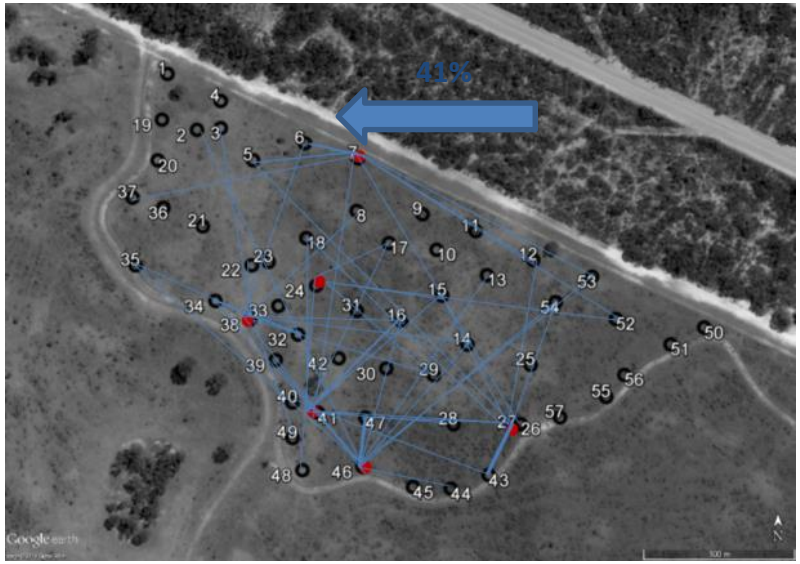
Monjebup A

- Evenly spaced
- At the edge of the restoration and adjacent Corackerup Nature Reserve

- Majority of pollen dispersal is in the shortest distance class ie 0-50m
- More closely tacking potential pollen dispersal classes

- High pollen immigration from outside 6ha = 41%
  - animals visiting from the reserve?



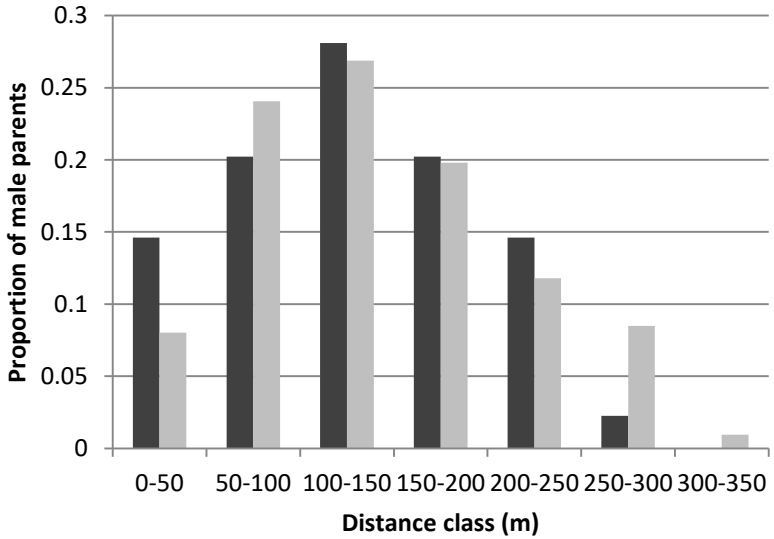


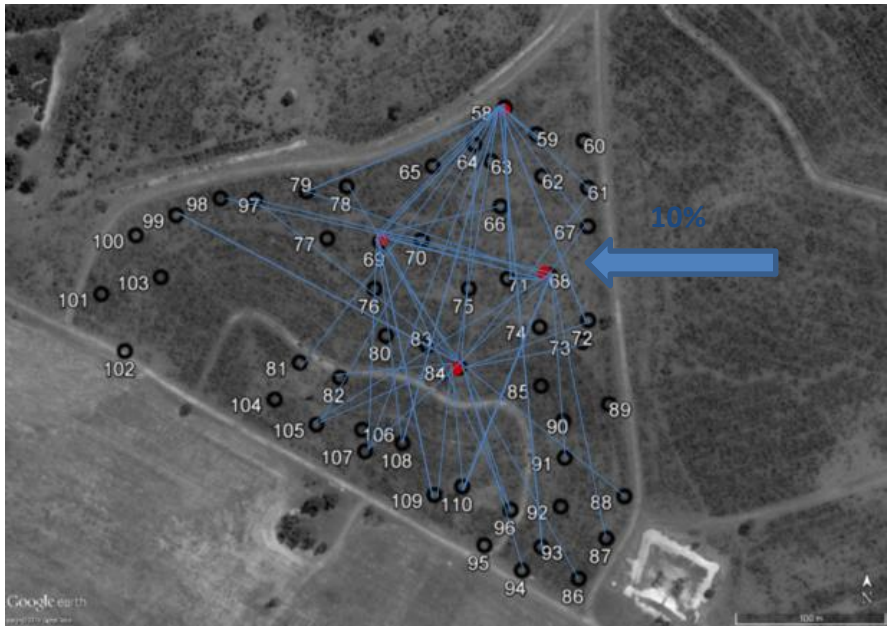
Pollen dispersal – *Banksia media*

Monjebup B

- Internal to the restoration planting 400m south
- More random pollen dispersal, closely tracking the potential pollen dispersal
- Majority of pollen dispersal is in the medium distance classes

- Lower pollen immigration from outside 7.6 ha = 10%



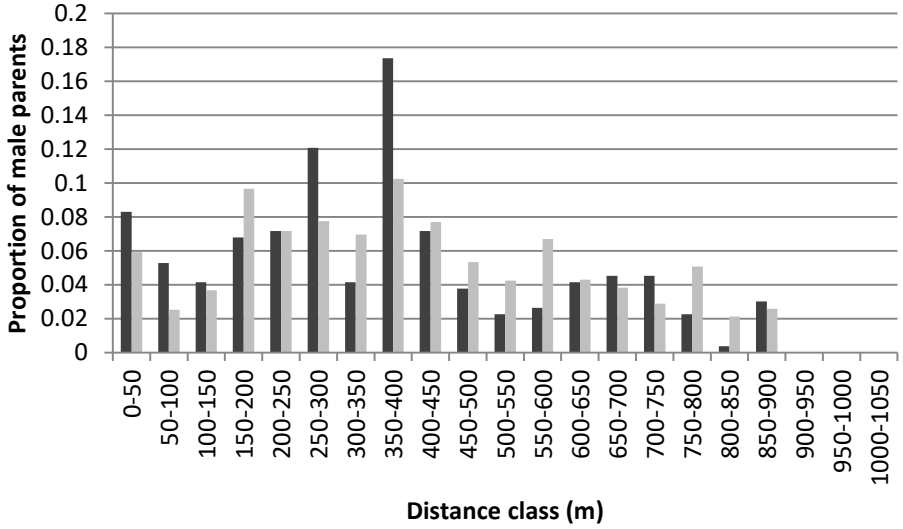


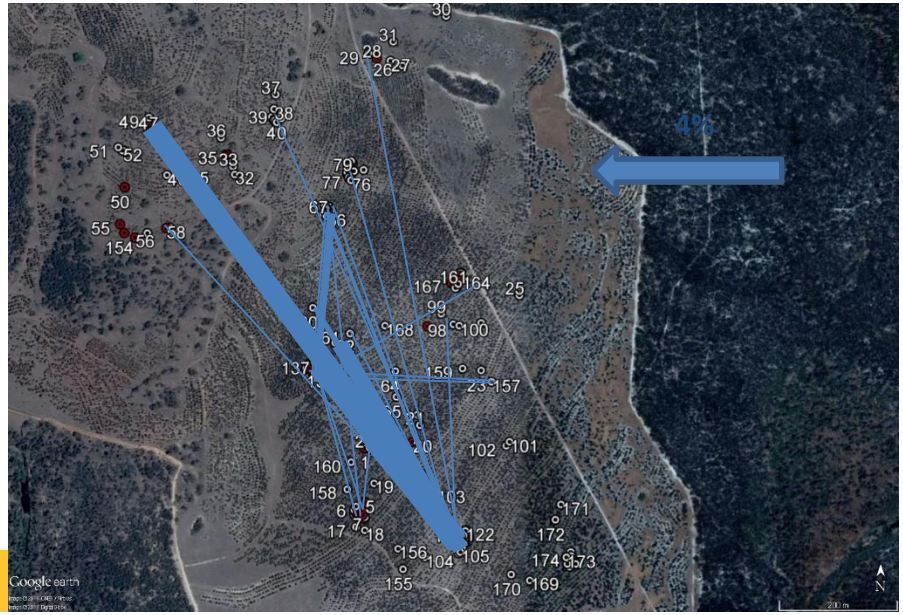
Pollen dispersal – *Hakea nitida*

Peniup

- Random pollen dispersal closely tracking potential pollen dispersal
- Were in nodes but lots of infill
- Long distance dispersal over 850m

- Low pollen immigration from outside 90ha = 4%





Is restoration working? II

- Is pollination resulting in comparable outcrossing rates?
  - Yes for insect pollinated species ☺
  - Reduced outcrossing may be some concern for Proteaceous species at Peniup?
    - Combined with low Ne for *B. media* and *H. nitida* and greater mating among relatives and low seed weight for *H. laurina*

- What are the patterns of pollen dispersal within restored populations?
  - Very different patterns for species and sites
  - Peniup – short distance dispersal for *B. media* but more random for *H. nitida*
  - Monjebup – much more random dispersal

- Is there pollen immigration into restoration populations from outside?
  - Yes ☺ Both Peniup and Monjebup have a degree of landscape connectivity at the broader scale
  - Very different levels depending on proximity to large nature reserve

Implications?

- Available levels of genetic diversity have been captured in restoration populations – sets them on a good future trajectory
- Diversity is of local provenance for fecund species - Proteaceous species may have been more widely sourced
- Plant pollinator interactions maintain mating systems for insect pollinated species
- Some limitations in Proteaceous species may be of concern – lower outcrossing rates and low number of effective pollen donors
  - Degree of inbreeding depression?
  - Impact on population fitness?
  - Mating systems are not static
- Rapid attractions of animal pollinators eg at Monjebup after 4 years
- Functional integration into the broader landscape

Where to from here?

- Importance of good seed source records eg to augment populations in the future
- Restoration sites could be seed production areas - *A. cyclops* and *M. acuminata*
- Ecological research and pollinator observations could inform on fecundity, pollinator or resource limitations - Proteaceous species
- Recruitment! Has to happen. Likely to require active management

Questions

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