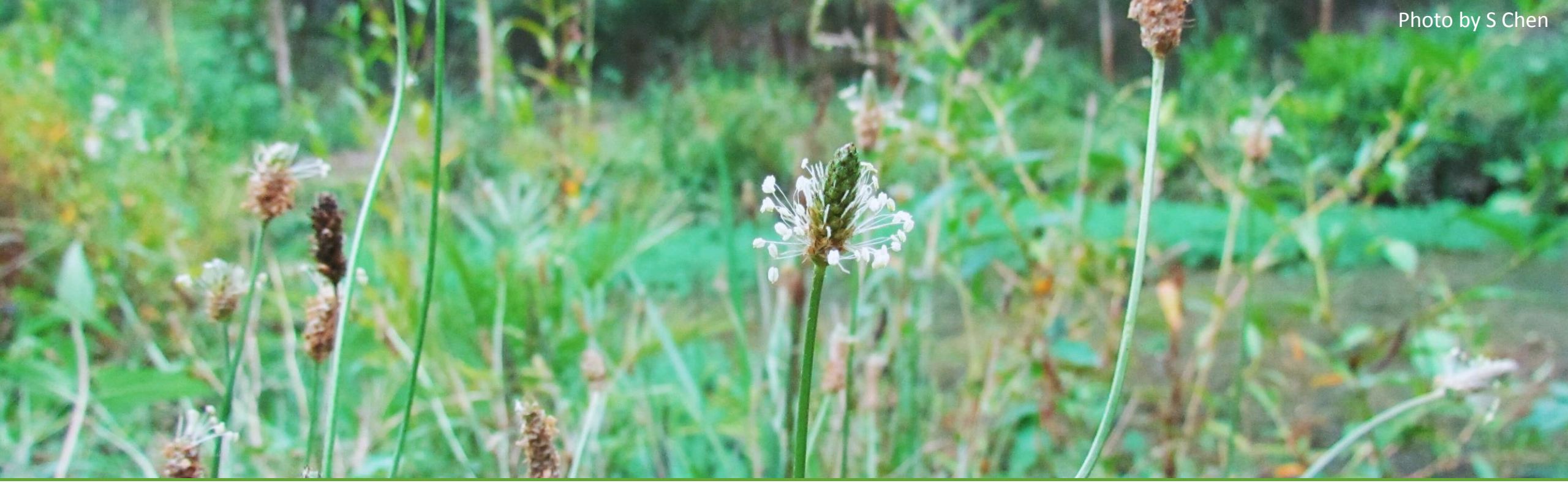


Population biology with *Plantago lanceolata* as a model system

A focus on genetics and seed ecology

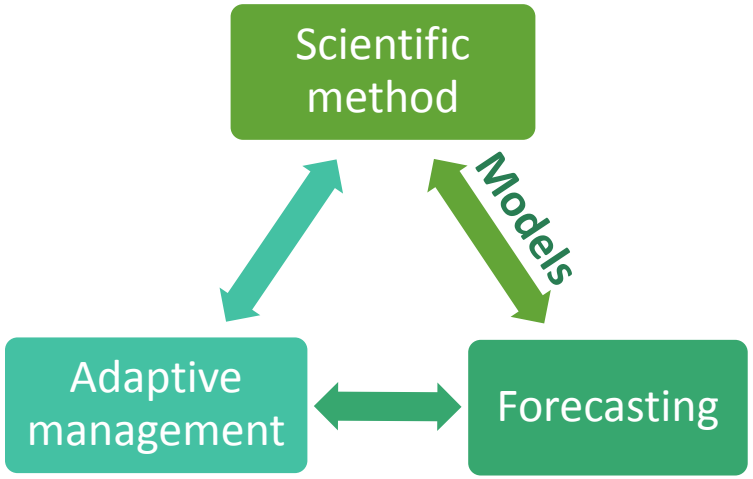
Stephanie H. Chen | Supervised by Prof. Glenda M. Wardle

Plant Pop Net

THE UNIVERSITY OF SYDNEY

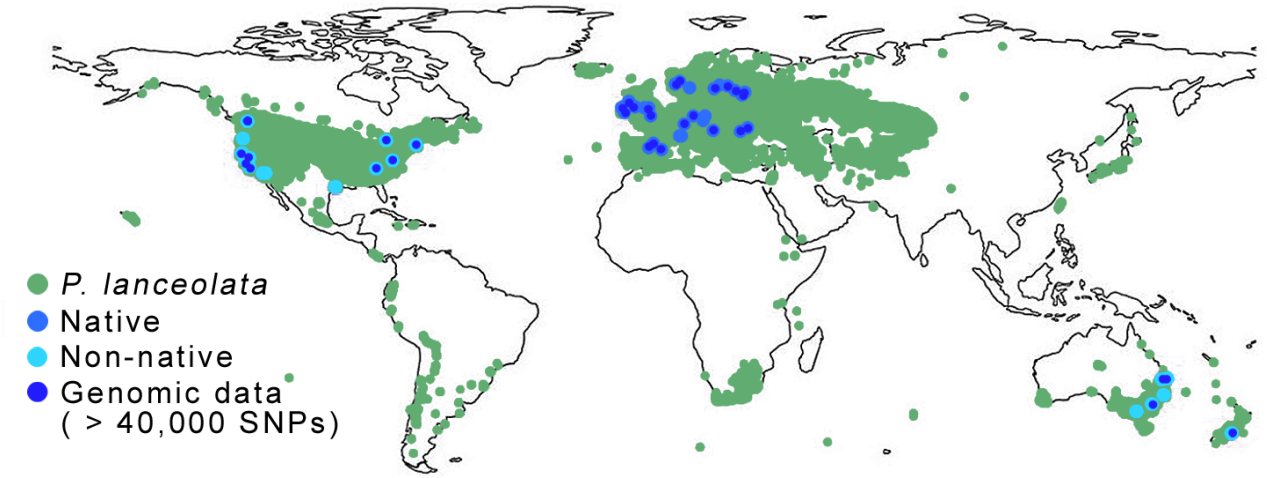
Ecological forecasting and PlantPopNet

- Ecological forecasting predicts changes in ecosystems and ecosystem components in response to an environmental driver.
- Lack of sampling within-species over large spatial extents → limited predictive capacity.



A spatially distributed model for population ecology

Plant Pop Net



53 sites across 16 countries

www.plantpopnet.com

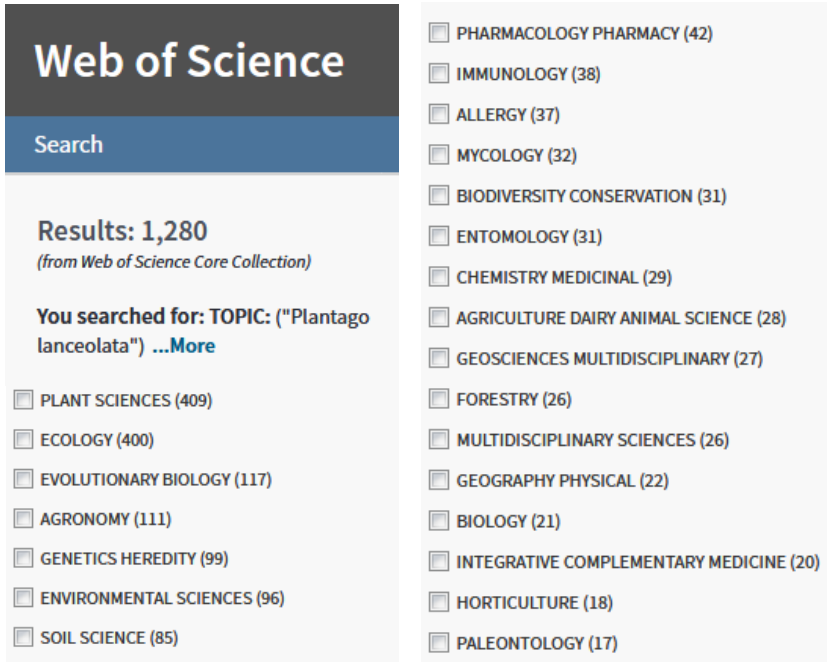
Plantago lanceolata L. (Plantaginaceae)

Ribwort plantain, narrowleaf plantain, English plantain, lamb's tongue; 'field-rat'

- Rosette-forming, facultative perennial herb.
- Native to Eurasia and introduced to Australia.
- Self-incompatible (Ross *Heredity*, 1973)
- Reproduces sexually and asexually via cloning (Primark *New Phytologist*, 1978).

Species	Family	Plant part	Use
Plantago lanceolata L.	Plantaginaceae	leaf	stuffed, pie, salad

Dogan et al. *Economic Botany*, 2004



Photos by S Chen

Plantago lanceolata L. (Plantaginaceae)

Ribwort plantain, narrowleaf plantain, English plantain, lamb's tongue; 'field-rat'

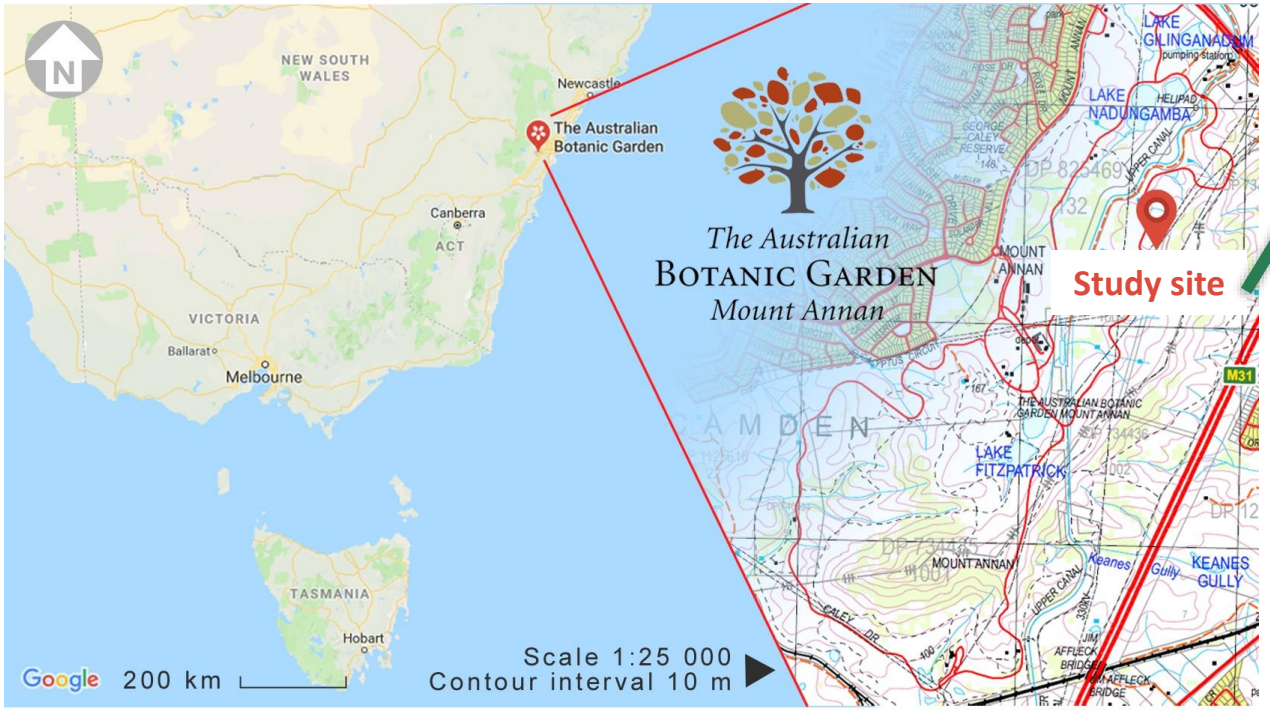
- Rosette-forming, facultative perennial herb.
- Native to Eurasia and introduced to Australia.
- Self-incompatible (Ross *Heredity*, 1973)
- Reproduces sexually and asexually via cloning (Primark *New Phytologist*, 1978).

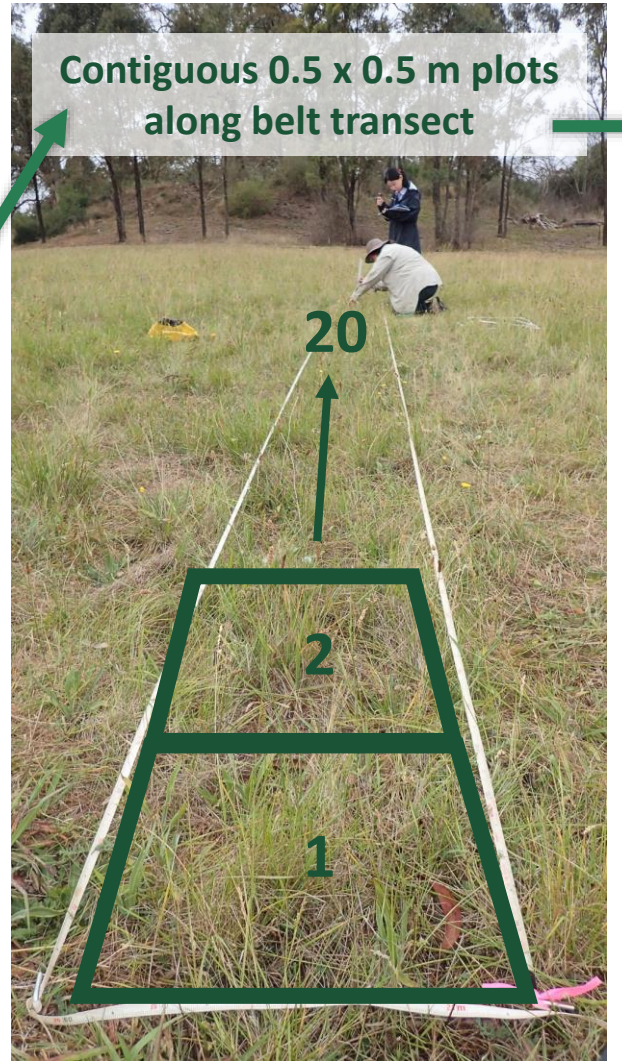
Assumptions of the model

1. No clonal reproduction.
2. No seed bank.

Do clonality and the seed bank matter for ecological modelling? How important is a reproduction framework for making predictions about population performance?

Study site – Mt Annan, NSW





4	4	6	86	1	0			no seeds
1	1	5	146	1	2	286	16	mature seed
1	1	5	146	1	2	227	19	flowering
1	1	6	110	2	5	425	25	seeds develo
1	1	6	110	2	5	372	26	flowering
1	1	6	110	27	5	360	20	flowering
1	1	6	110	27	5	380	MISSING	MISSING
1	1	5	64	6	0			no seeds
4	2	7	65	9	0			no seeds
4	3	3	61	6	0			no seeds
4	4	5	84	10	1	205	9	seeds develo
1	1	4	91	5	0			no seeds
1	1	6	97	8	0			no seeds
2	1	6	99	17	2	235	10	flowering
2	1	6	99	17	2	250	9	flowering

Demographic surveys

Size (no. leaves)
Growth
Survival

Photos by G Wardle and B Tamayo

Part 1 Spatial analyses and clonality

Aim

Examine the spatial patterns of clonality in *P. lanceolata* at a fine-scale at the population level and relate the extent of clonality to level of plant density.

> Plants stand still and wait to be counted.
>
> Harper, *Population Biology of Plants*, 1977

Spatial point patterns in ecology

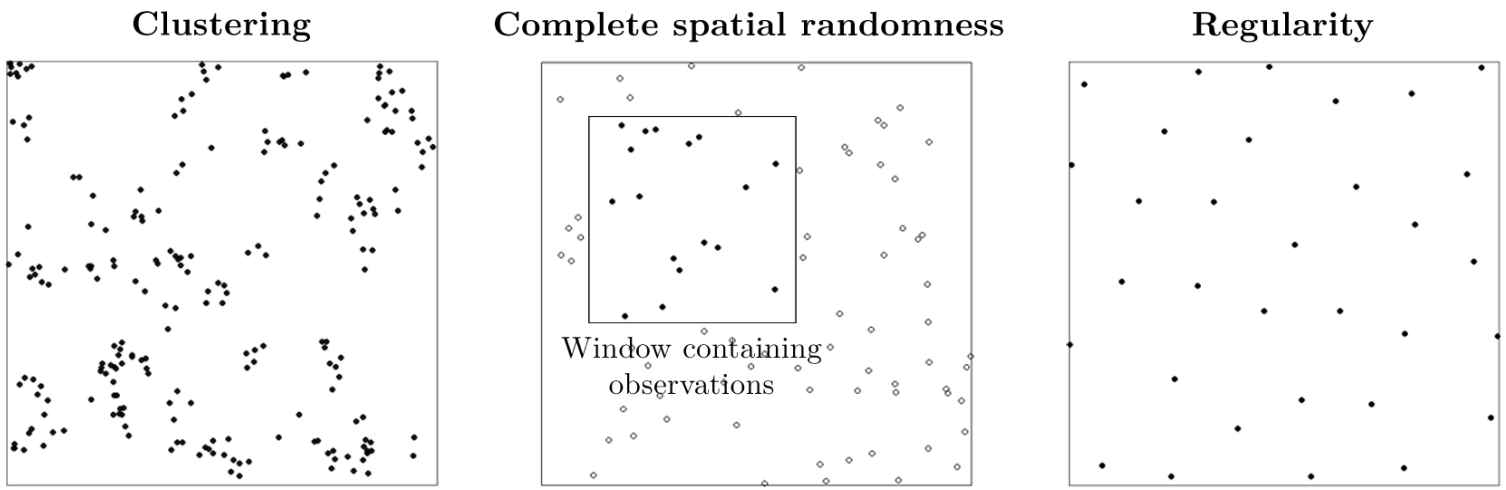


Photo by G Wardle

Part 1 Spatial analyses and clonality

Aim

Examine the spatial patterns of clonality in *P. lanceolata* at a fine-scale at the population level and relate the extent of clonality to level of plant density.

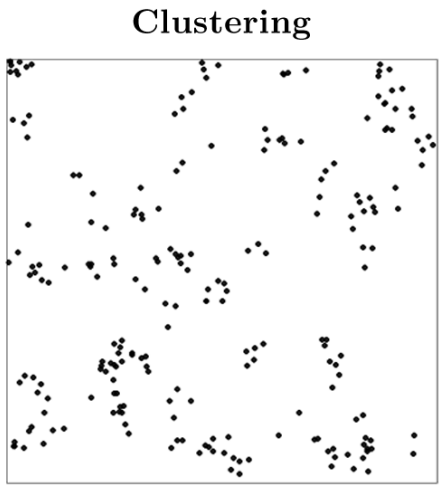
> Plants stand still and wait to be counted.
>
> Harper, *Population Biology of Plants*, 1977

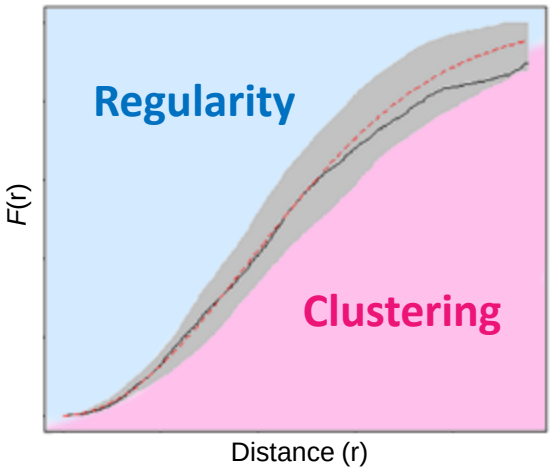
Spatial point patterns in ecology

First order: Empty space function *F* and *G* function

Second order: Ripley's *K* and Beslag's *L*

spatstat





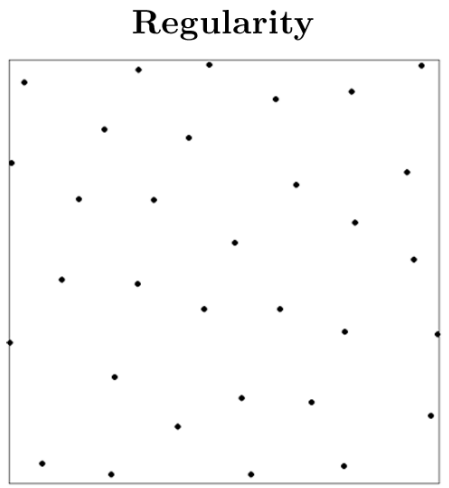
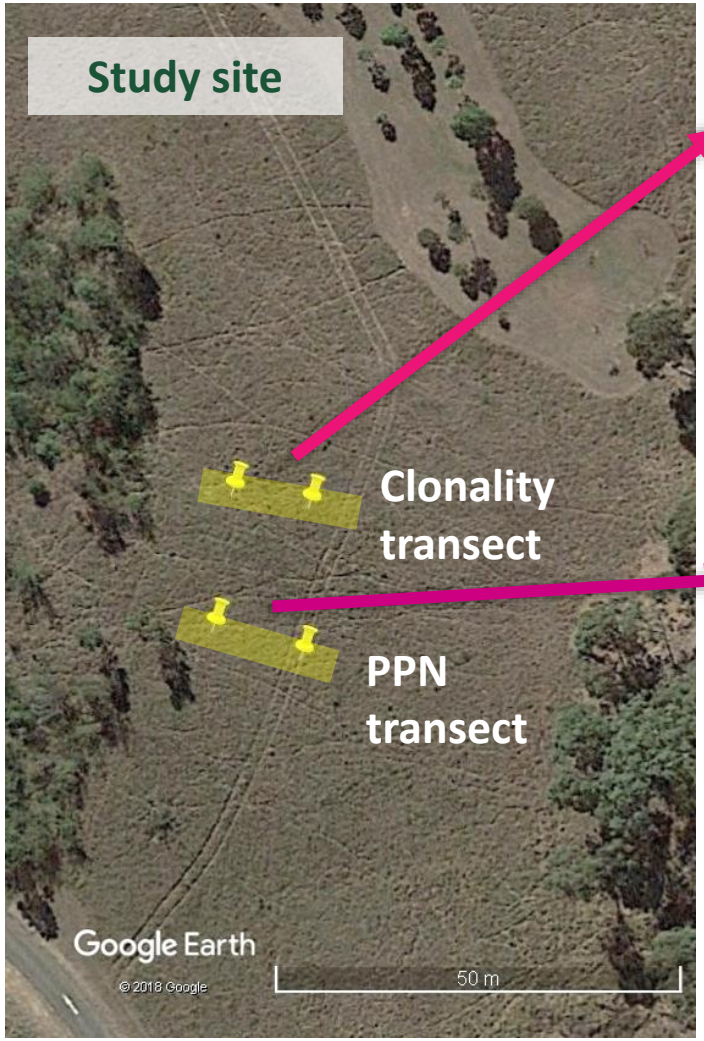
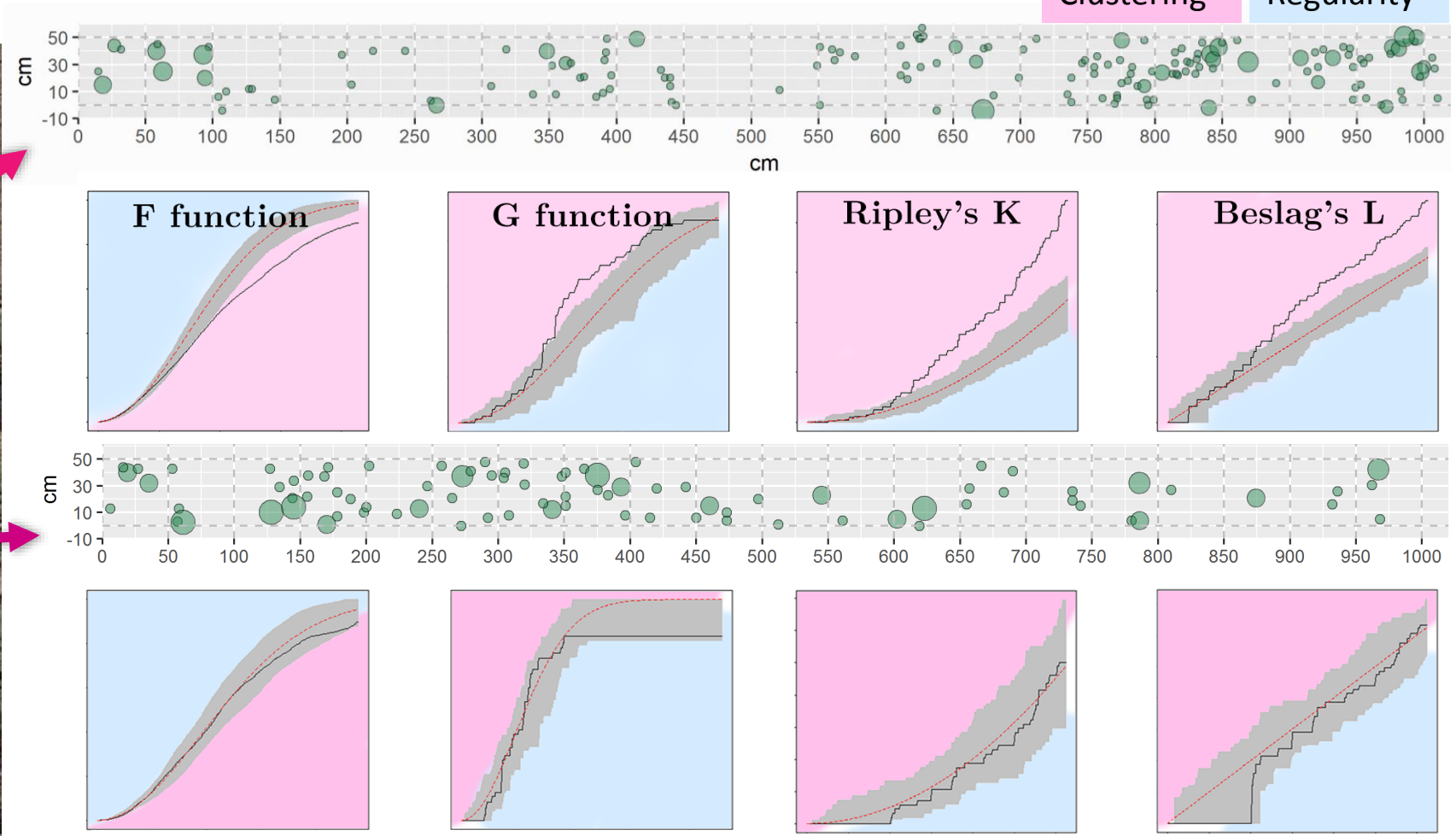


Photo by G Wardle

Part 1 Spatial analyses and clonality

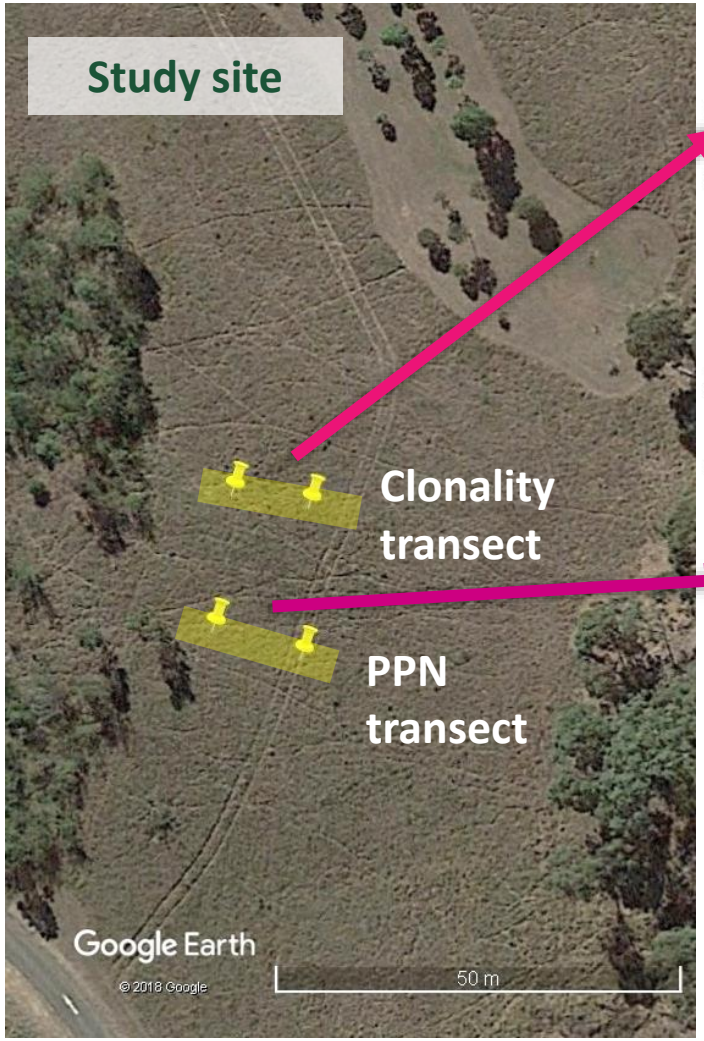


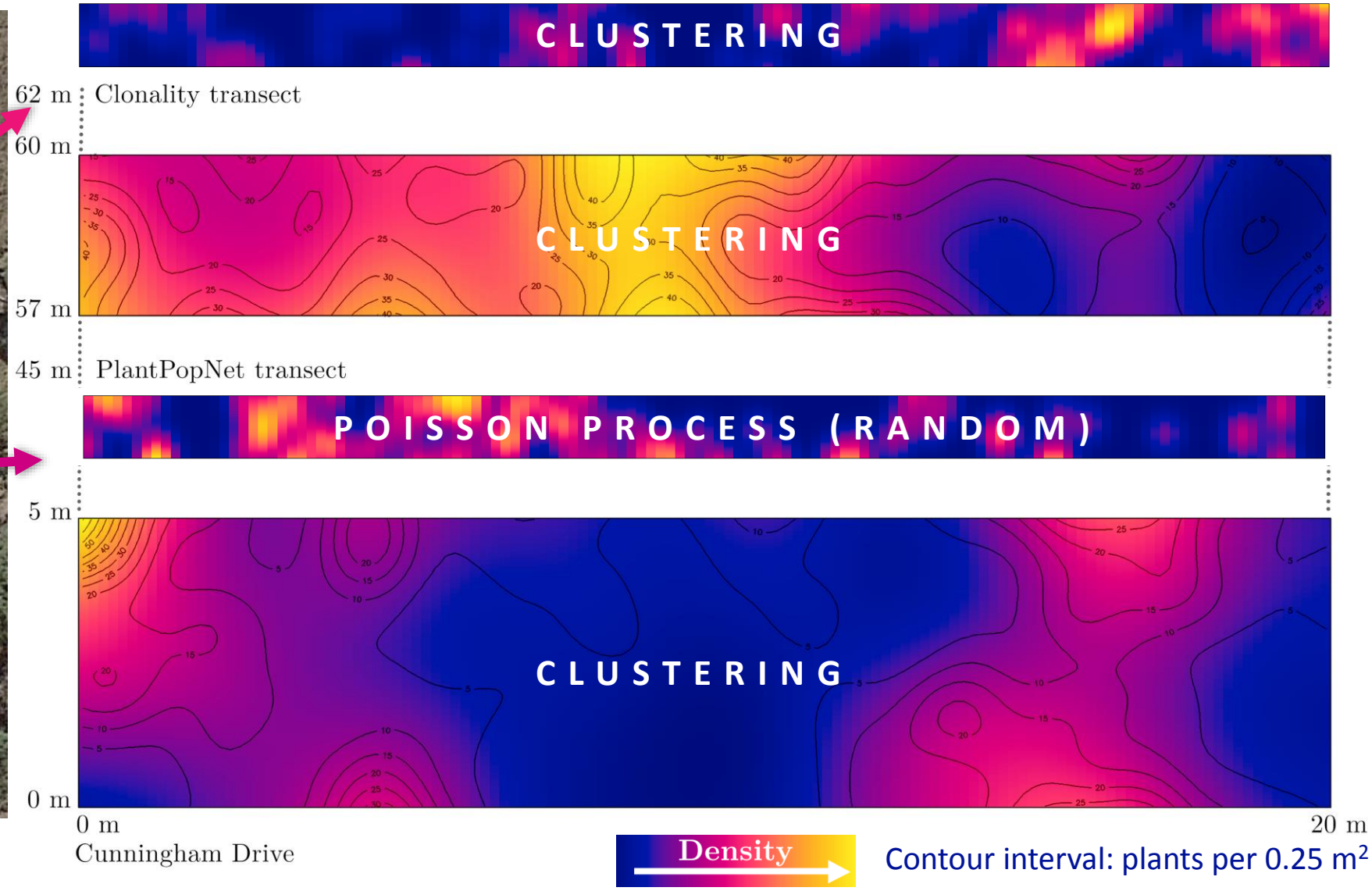
Clustering | Regularity



Part 1 Spatial analyses and clonality

Clustering | Regularity





Contour interval: plants per 0.25 m^2

Part 1 Spatial analyses and clonality

Above ground observations

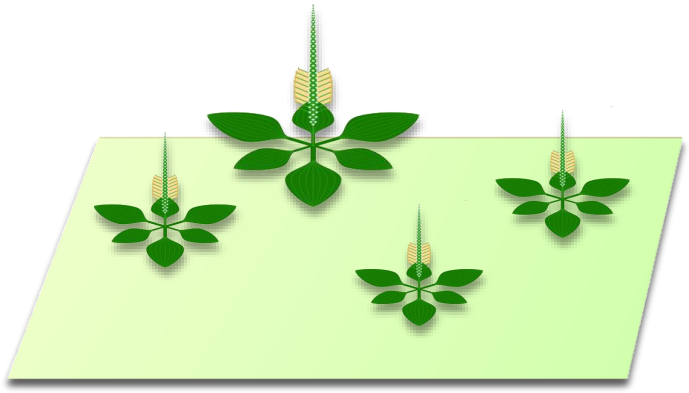
Note suspected clones

Below ground observations

Excavate all plants

Collect leaf samples

4 individual plants = 4 genets



Photos by G Wardle and S Chen

Part 1 Spatial analyses and clonality

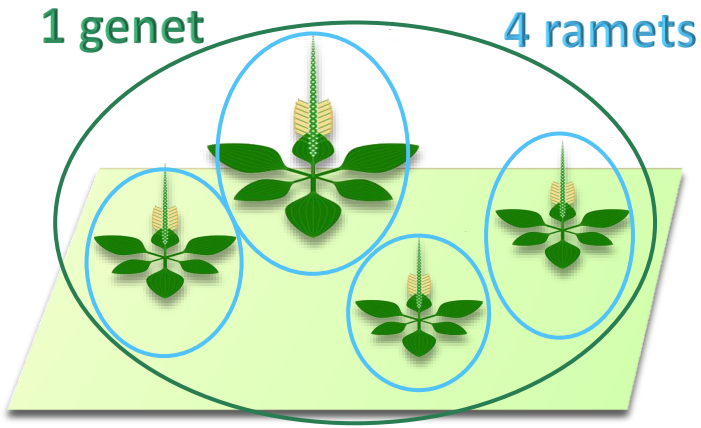
Above ground observations

Note suspected clones

Below ground observations

Excavate all plants

Collect leaf samples



Photos by G Wardle and S Chen

Part 1 Spatial analyses and clonality

Above ground observations

Note suspected clones

Below ground observations

Excavate all plants

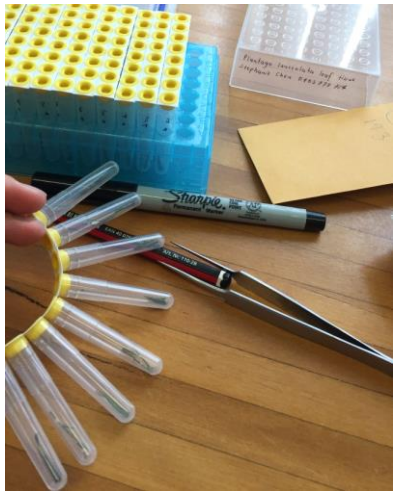
Collect leaf samples

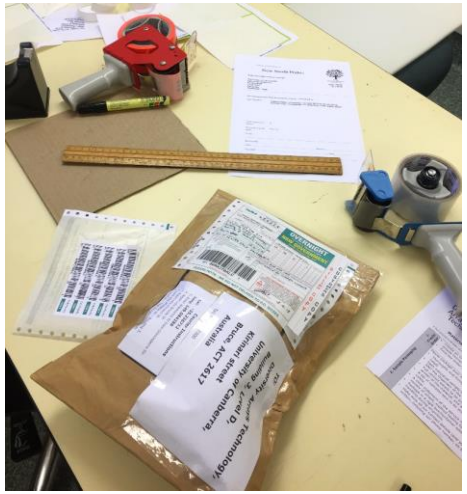
Extraction and sequencing (DArTseqLD)

Filter and analyse SNPs in R

10630 SNP markers
1747 after filtering

Extent (%) clonal
Map spatial patterns





Diversity Arrays Technology

282 samples (putative genets)

AlleleID	AlleleSeq	TrimmedS	Chrom_Ba	ChromPos	AlnCnt_Ba	AlnEvalue	Chrom_Fu	ChromPos	AlnCnt_Fu	AlnEvalue	SNP	SnpPositi	103_1	173ros1_9	181_113	189_122	109_18	117_32	125_42	133_53	141_61	149_71	157_79	165_87	104ros1_2	174_106	182_114	190_123
23393541	TGCAGAA	TGCAGAAGAGCAAA		0	0	999		0	0	999	8:A>T	8	0	-	0	0	0	-	0	0	0	0	-	-	0	0	0	-
23403936	TGCAGGA	TGCAGGAGCTATTTT		0	0	999		0	0	999	20:C>T	20	0	2	0	0	0	0	0	0	0	2	-	0	-	0	0	2
23402795	TGCAGGT	TGCAGGTGAAGATAT		0	0	999		0	0	999	60:T>C	60	1	2	0	2	1	0	0	1	1	1	2	1	1	0	-	0
34806946	TGCAGGT	TGCAGGTTCAGTCAA		0	0	999		0	0	999	37:A>C	37	-	0	0	0	1	-	1	-	1	0	0	-	-	2	0	2
23415248	TGCAGCT	TGCAGCTTTATGGAA		0	0	999		0	0	999	50:C>A	50	-	-	-	0	-	0	-	0	0	-	-	-	-	-	-	-
23422620	TGCAGTT	TGCAGTTGGGATACT		0	0	999		0	0	999	26:T>G	26	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
28861238	TGCAGTT	TGCAGTTTAGCTAGG		0	0	999		0	0	999	17:A>T	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34806979	TGCAGTT	TGCAGTTTCATCTCTG		0	0	999		0	0	999	9:A>T	9	-	1	2	1	1	1	-	0	2	0	-	0	-	-	-	1
23402048	TGCAGCT	TGCAGCTACAAGTCT		0	0	999		0	0	999	25:C>T	25	0	0	0	-	-	0	0	2	0	0	-	0	0	0	-	0
23415277	TGCAGGG	TGCAGGGGAAAACA		0	0	999		0	0	999	56:T>A	56	0	-	-	-	0	-	-	-	0	-	-	-	0	2	-	0
100003687	TGCAGGT	TGCAGGTTTAGCCTT		0	0	999		0	0	999	34:T>A	34	-	-	0	0	0	0	-	0	0	-	0	0	-	0	0	-	0
23394248	TGCAGCA	TGCAGCAAATATGTA		0	0	999		0	0	999	45:C>T	45	2	0	2	2	0	0	0	2	0	0	0	0	2	0	0	0
23396835	TGCAGTT	TGCAGTTTGTAAATC		0	0	999		0	0	999	56:G>A	56	-	-	-	0	0	0	-	0	0	0	1	0	-	-	-	0
34806380	TGCAGAA	TGCAGAAAAATTATT		0	0	999		0	0	999	53:A>G	53	-	0	0	-	-	0	-	-	-	0	-	0	-	0	0	0
23420907	TGCAGAA	TGCAGAAAACAACAT		0	0	999		0	0	999	30:C>A	30	0	0	-	0	0	0	-	-	-	0	1	-	0	0	-	-	-
23407039	TGCAGAA	TGCAGAAAACAATAC		0	0	999		0	0	999	19:T>C	19	-	-	-	1	0	-	1	-	-	-	-	1	-	-	1	-	1
23393355	TGCAGAA	TGCAGAAAACAGGC		0	0	999		0	0	999	47:C>A	47	0	0	-	-	-	-	-	-	-	-	0	0	0	-	-	0	-
34806384	TG			0	0	999		0	0	999	35:G>A	35	2	0	2	0	0	0	0	0	0	2	2	2	2	0	0	0
23400640		AA	TGCAGAAAAC							999	68:T>G	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
348049		AGAA								999	16:T>G	16	0	0	0	0	0	-	0	0	0	0	0	0	0	0	-	0
372110		CAGAA								999	36:G>A	36	0	0	0	0	0	0	-	0	0	-	0	0	0	0	0	0
234070		AGAA								999	14:A>G	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23389379										999	23:C>T	23	1	-	1	1	0	-	-	-	-	1	0	0	1	1	1	1
34806386	TGCAGA									999	43:G>A	43	0	0	0	1	2	0	0	2	0	0	1	0	0	0	0	0
100003725	TGCAGAA									999	49:G>A	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23389159	TGCAGAA	TGCAGAAAATCATGA		0	0	999		0	0	999	46:G>T	46	-	0	1	1	0	-	-	-	-	1	-	-	-	-	0	0
23385615	TGCAGAA	TGCAGAAAATTGATG		0	0	999		0	0	999	15:A>C	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

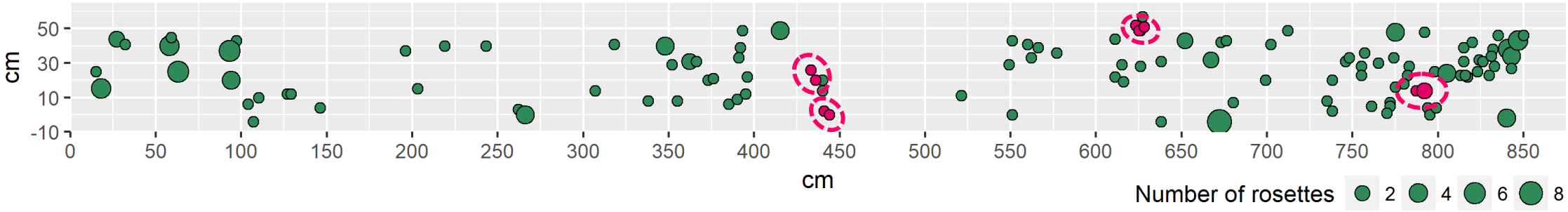
dartR
SNPRelate

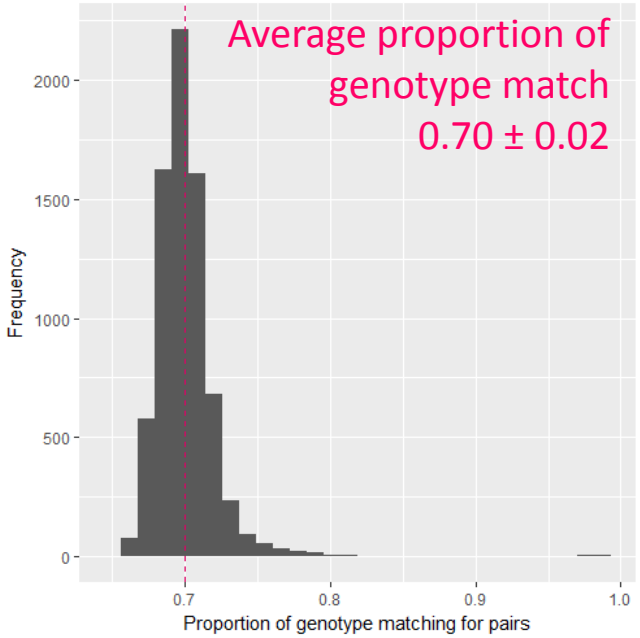
SNP: single nucleotide polymorphism

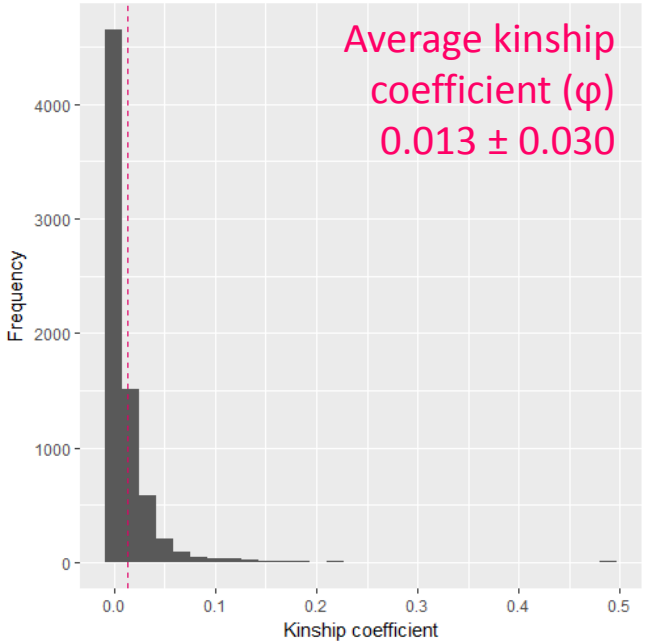
Animation by Marc Christoforidis; Photos by S Chen

Part 1 Spatial analyses and clonality

Clones – genotype match > 95 % and kinship coefficient > 0.45 (1576 SNPs)







- 4 clone groups
- 121 samples representing putative genets (plants)
- 165 ramets represented

Clones are present
By plant (genet): 8.3 %
Ramet: 6.7 %

Part 1 Spatial analyses and clonality

MACD A

Areas of high plant density near clonality transect

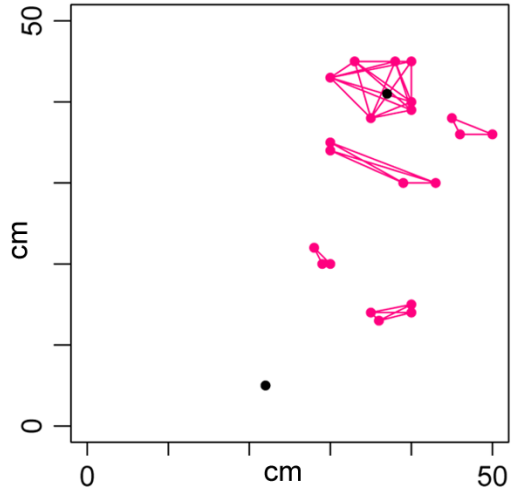
MACD C

Duckpond A

Duckpond B

Pilot study (Dublin, Ireland)

- 1500 SNPs
- 17 putative genets
- 23 ramets represented
- Genet: 82 %
- Ramet: 91 %

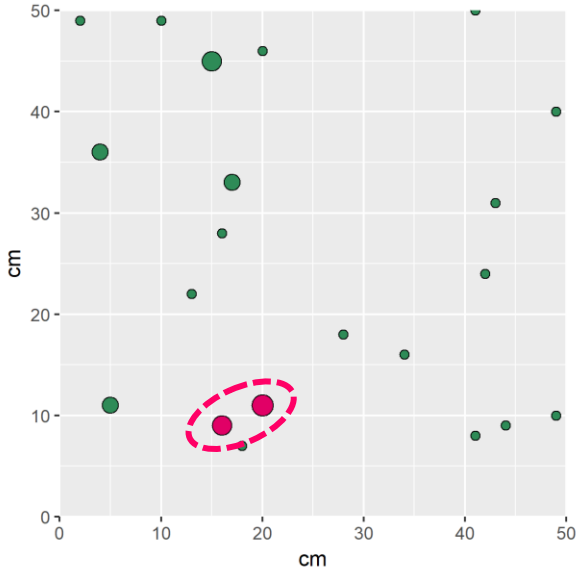


Smith and Buckley (unpublished)

Photos by G Wardle

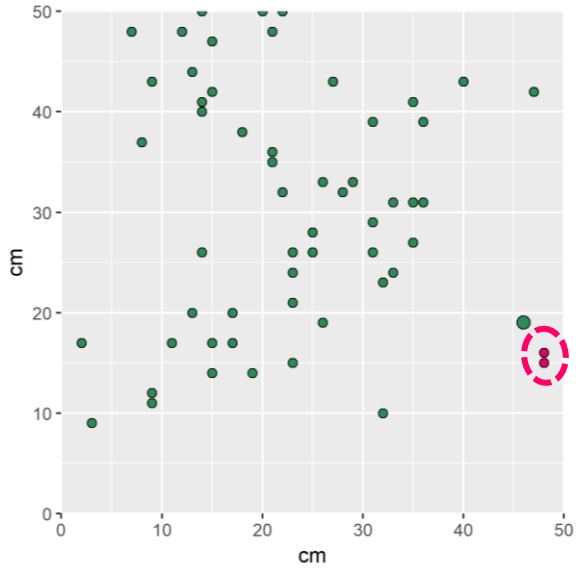
Part 1 Spatial analyses and clonality

MACD A



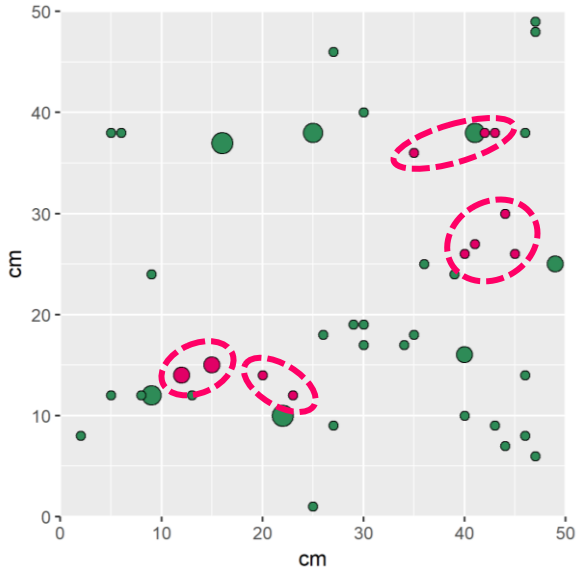
- 1280 SNPs
- 21 putative genets
- 31 ramets represented
- Genet: 9.5 %
- Ramet: 23 %

MACD C



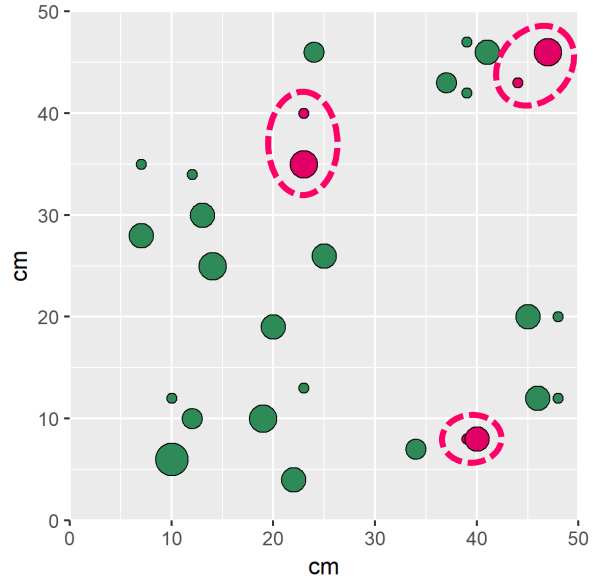
- 1386 SNPs
- 57 putative genets
- 58 ramets represented
- Genet: 3.5 %
- Ramet: 3.4 %

Duckpond A



- 1327 SNPs
- 46 putative genets
- 62 ramets represented
- Genet: 24 %
- Ramet: 21 %

Duckpond B



- 1240 SNPs
- 29 putative genets
- 68 ramets represented
- Genet: 21 %
- Ramet: 21 %

Relatively weak correlation between plant density and extent of clonality

Adjusted R^2 = 0.39, n = 21 plots

Part 2 Seed ecology

Collect seeds from plants in field

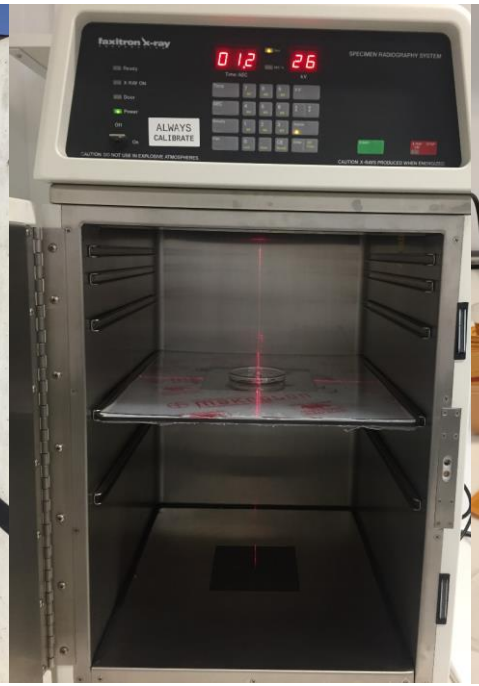
Measure functional traits of seeds
(n = 1493 from 51 plants)

Test seed viability and compare with commercial seed stock

X-ray	Ex-situ germination experiment

Aim

Investigate the seed bank and relate functional traits to seed viability. Quantify the number of seeds in the soil seed bank and relate this to plant density.



Photos by S Chen

Part 2 Seed ecology

Collect seeds from plants in field

Measure functional traits of seeds
(n = 1493 from 51 plants)

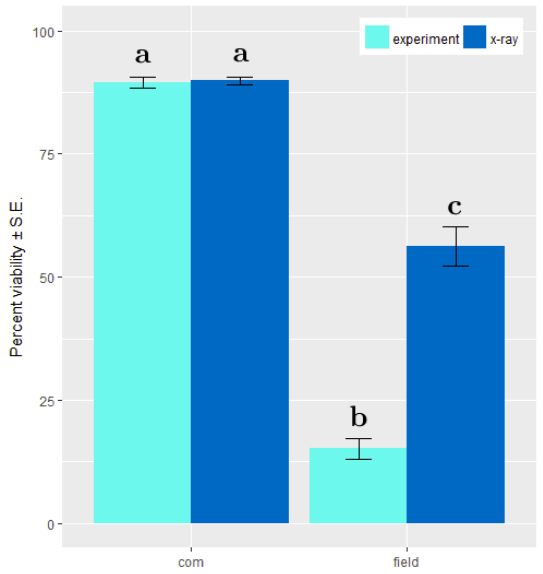
Test seed viability and compare with commercial seed stock

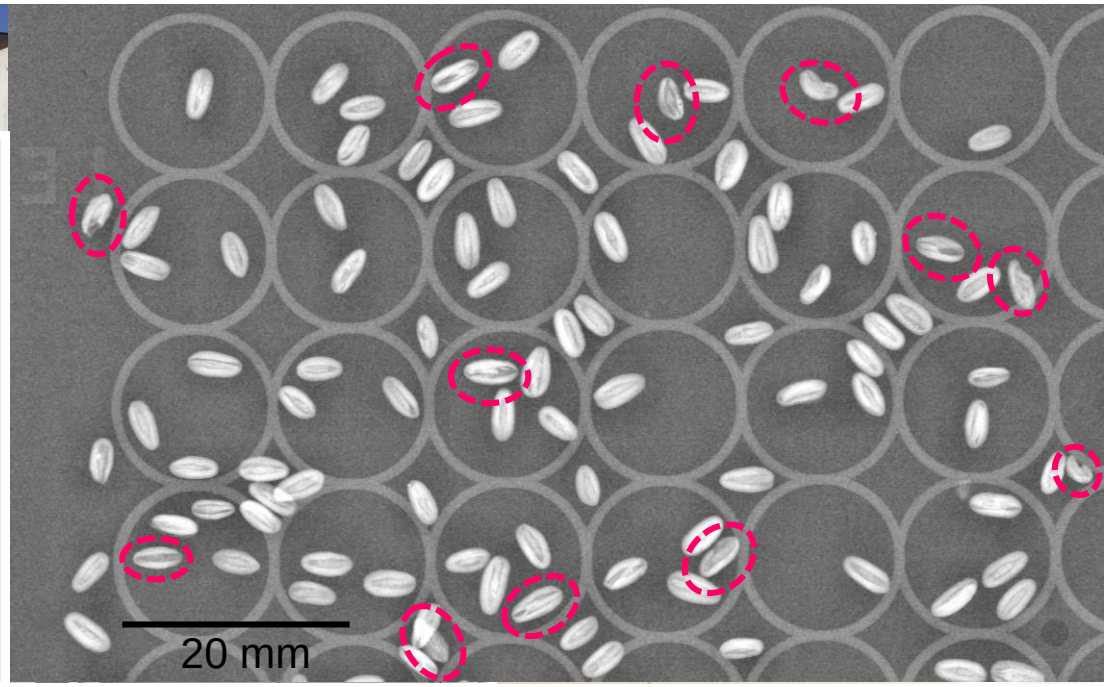
X-ray	Ex-situ germination experiment

25 °C, 16 h photoperiod for 26 days

29.3 seeds per inflorescence head

Seed dormancy





Photos by S Chen

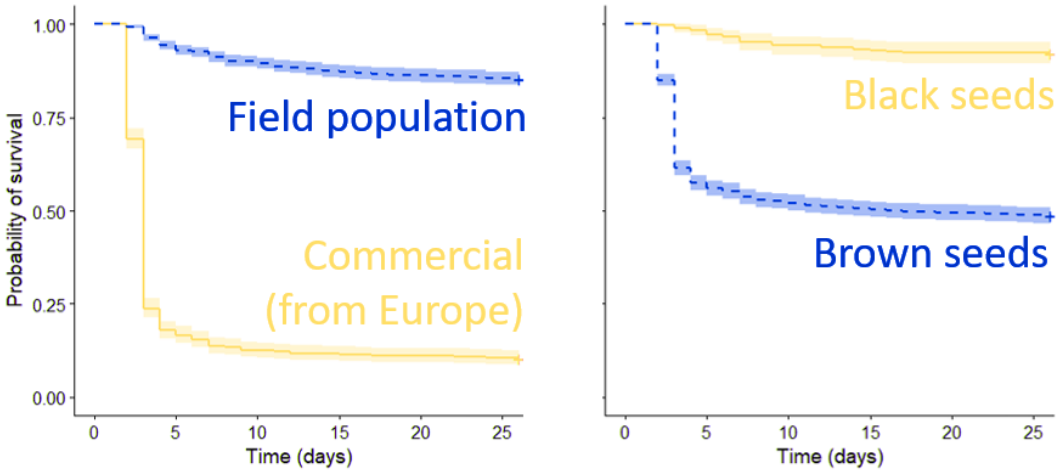
Part 2 Seed ecology – survival analysis

survival
coxme

- Brown seeds have higher mass and higher probability of germination than black seeds.
- Commercial seeds 89.5 % germination (n = 1039) which was significantly higher than the field population (1493 seeds collected from 51 plants)

14.7 % germination

Kaplan-Meier survivor curves



Cox proportional hazards model

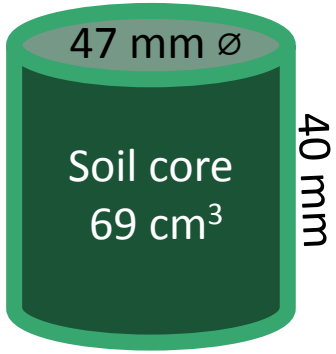
$$h(t) = h_0(t)\, e^{(\beta_{OriginField}x + \beta_{ColourBrown}x + \beta_{OriginField:ColourBrown}x + \text{lot as random effect} + \varepsilon)}$$

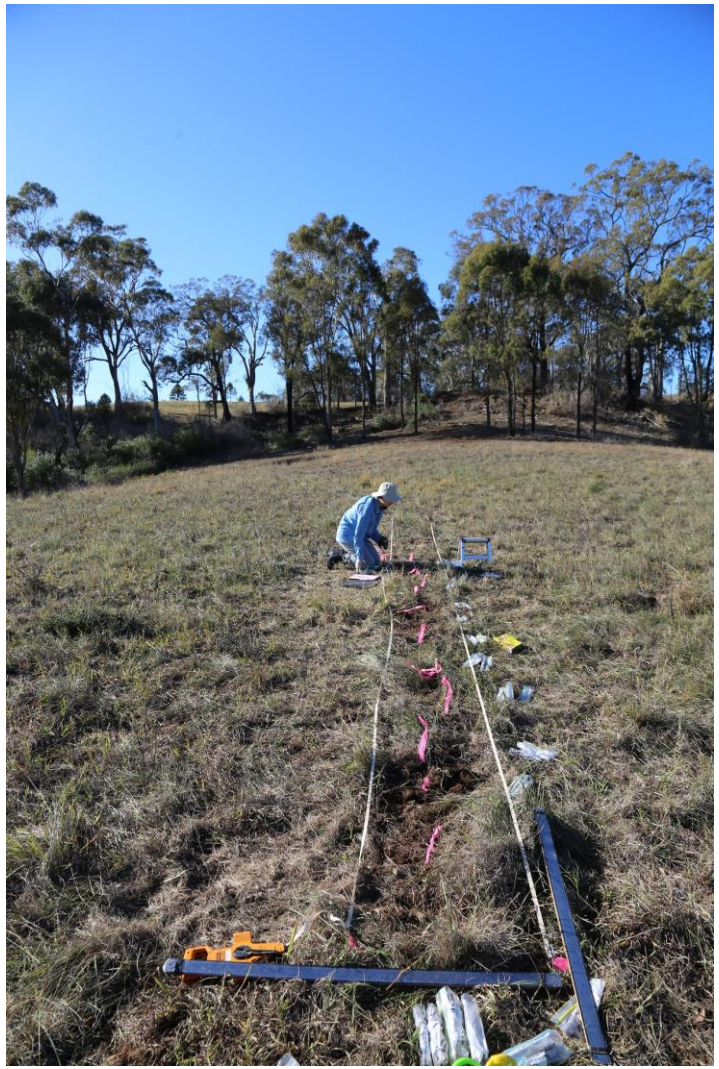
Factor	Coefficient	HR [95 % CI]
Origin		
Commercial	0	1 (reference)
Field	-0.9628	0.382 [0.1329, 1.0968]
Colour		
Black	0	1 (reference)
Brown	3.807***	45.02 [24.72, 81.99]
Interaction		
Commercial:Black	0	1 (reference)
Field:Brown	-2.426***	0.008837 [0.03952, 0.19763]

***P < 0.001

Part 2 Seed ecology – soil seed bank

- Soil sampling in each plot of clonality transect
- 20 samples total – 70 seeds and 11 seedlings
- Majority of seeds in top 2 cm of soil





Photos by G Wardle

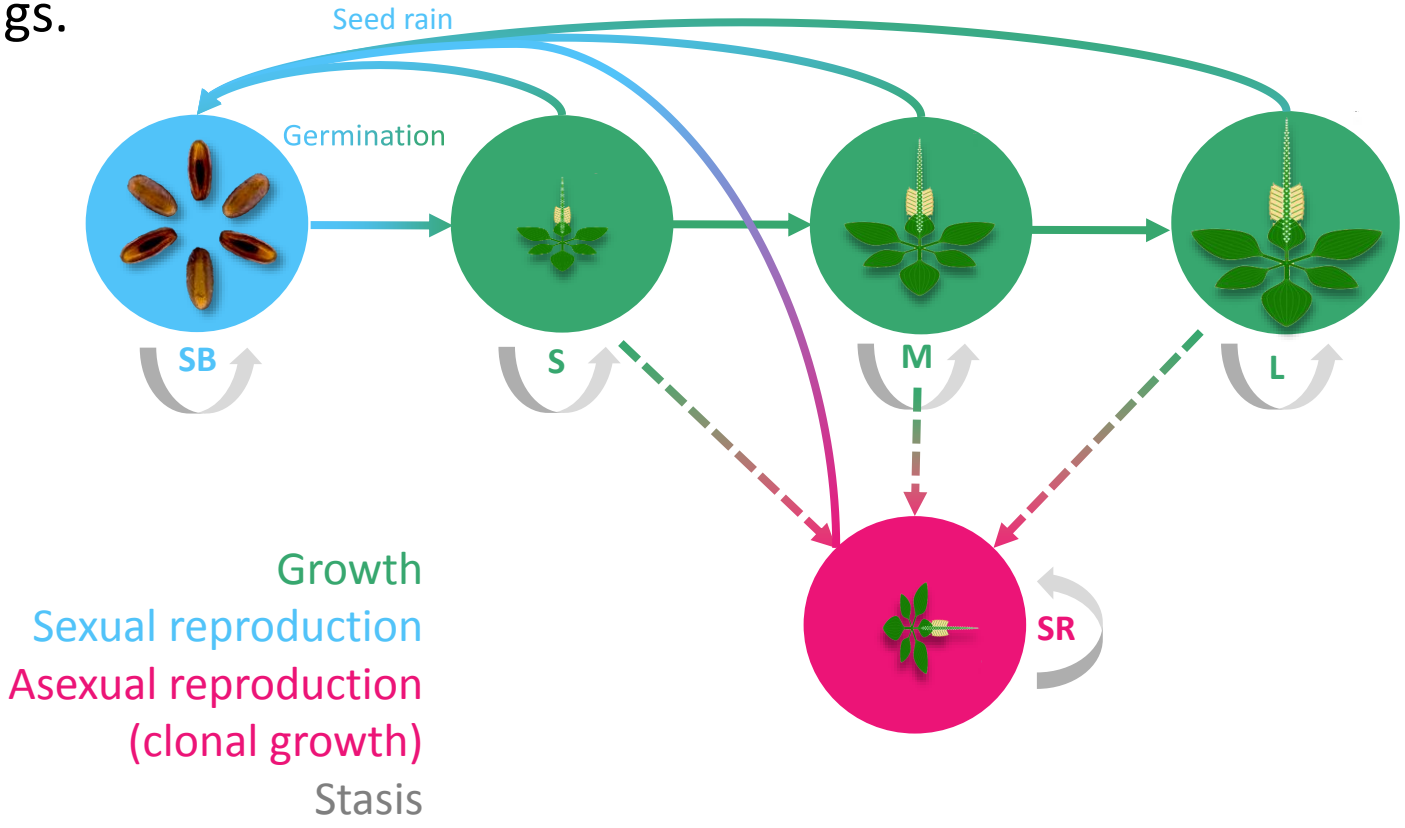
Part 3 Bringing it all together – population modelling

Aim

Integrate clonality and seed bank findings into the parameterisation of the population model and describe the effect on demographic parameters. Develop recommendations for PlantPopNet data collection and modelling based on findings.

5 bins

Matrix population models (Caswell 1988, 2001) quantify ways individuals survive and reproduce.



Life-cycle graph of *Plantago lanceolata*

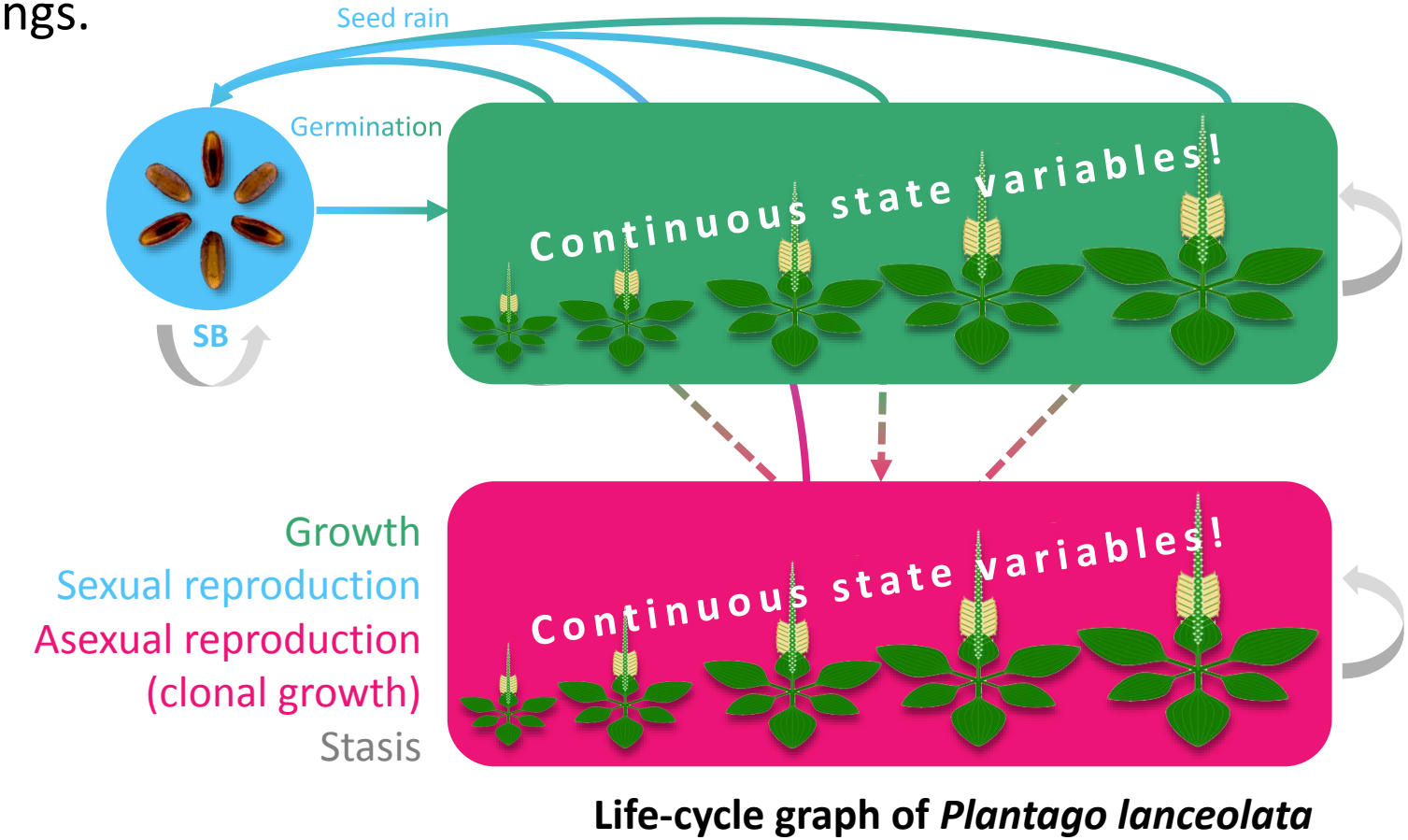
Part 3 Bringing it all together – population modelling

Aim

Integrate clonality and seed bank findings into the parameterisation of the population model and describe the effect on demographic parameters. Develop recommendations for PlantPopNet data collection and modelling based on findings.

Matrix population models (Caswell 1988, 2001) quantify ways individuals survive and reproduce.

Integral projection models (IPMs; Easterling et al. 2000, Ellner and Rees 2006) are more appropriate for continuous state variables



Life-cycle graph of *Plantago lanceolata*

Part 3 Bringing it all together – population modelling

- Integral projection models (IPMs) are defined by a kernel, *K*.

IPMpack

$$n_{t+1}(z') = \int_\Omega \boldsymbol{K}(z) n_t(z) dz = \int_\Omega [\boldsymbol{P}(z', z) + \boldsymbol{C}(z', z) + \boldsymbol{F}(z', z)] n_t(z) dz$$

$$\boldsymbol{P}(z', z) = \text{survive}(z)\ \text{growth}(z', z)$$

$$\boldsymbol{C}(z', z) = \text{clone}(z)\ \text{clonesNext}(z')\ \text{cloneSize}(z')$$

$$\boldsymbol{F}(z', z) = \underbrace{\text{fec0}(z)\ \text{fec1}(z)\ \text{fec2}\ (1 - \text{goSB})\ \text{fec3}\ \text{fec4}\ \text{recruitSize}(z')}_{\text{direct recruits}} +$$

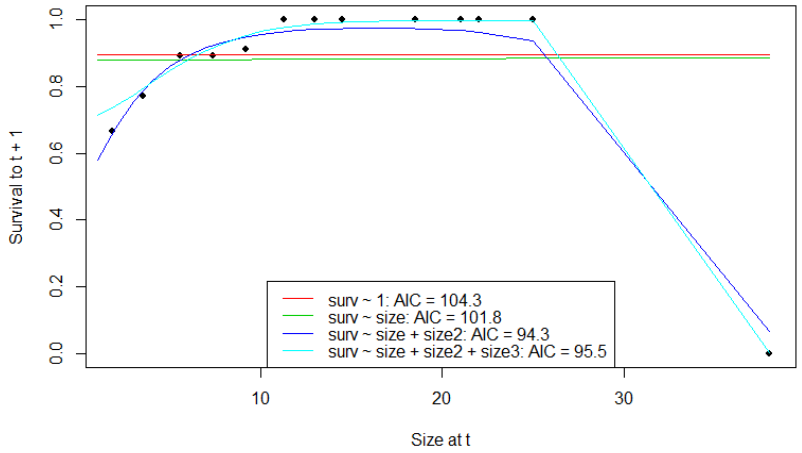
$$\underbrace{\text{fec0}(z)\ \text{fec1}(z)\ \text{fec2}\ \text{goSB}\ (1 - \text{staySB})\ \text{fec3}\ \text{fec4}\ \text{recruitSize}(z')}_{\text{recruits from the seed bank}}$$

Size at t and (t + 1)
Survival (7.5 month timestep)

8.3 % clonality (genet)
6.7 % clonality (ramet)

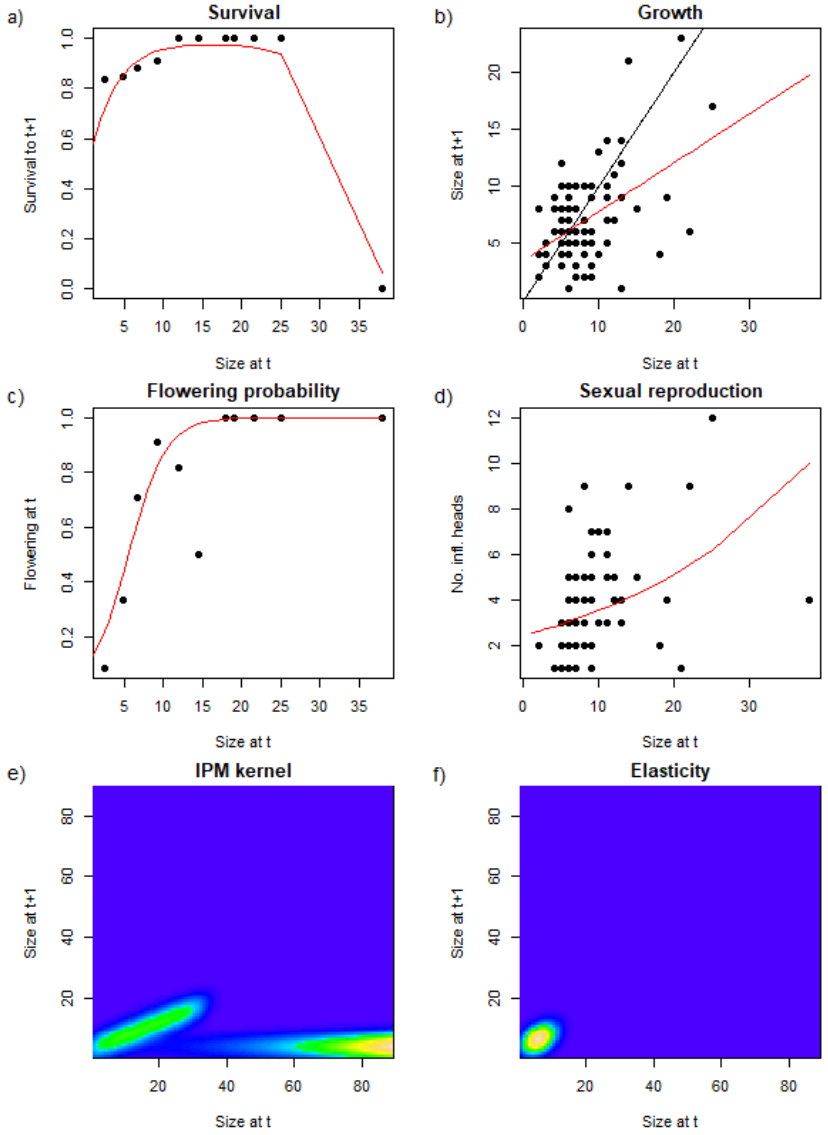
14.7 % germination
fec2 = 29.3 seeds per inflorescence

Part 3 Population modelling – key outputs



Parameterisation using vital rate regressions

Part 3 Population modelling – key outputs



$N_{t+1} = N_t + Births – Deaths + Immigrants – Emigrants$

$Population\ growth\ rate\ (\lambda) = N_{t+1}/N_t$

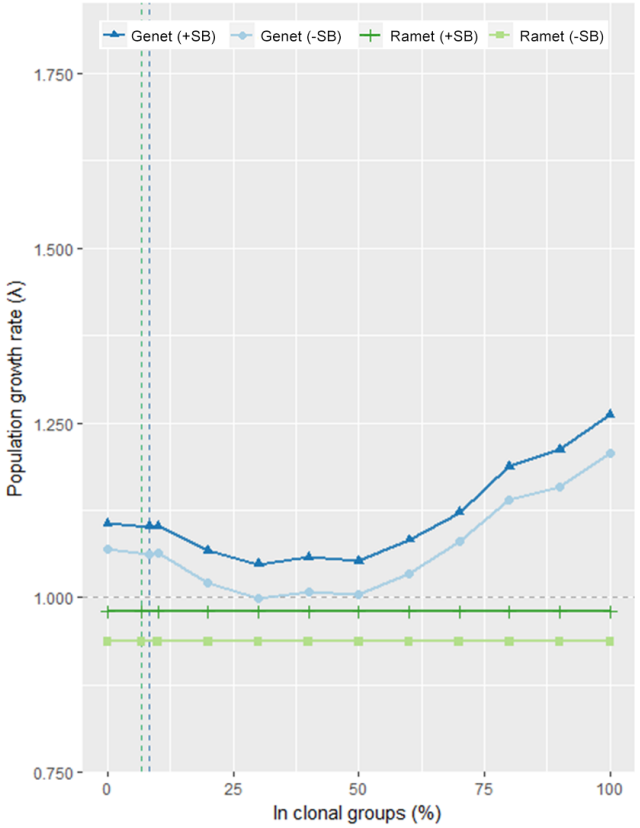
$\lambda > 1$ Population increasing $\lambda = 1$ Stable $\lambda < 1$ Population decreasing

	101 plants and 13 seedlings	+ seed bank	- seed bank
BY GENET	**+ clonality (8.3 %)**	1.102	1.063
	- clonality	1.106	1.069

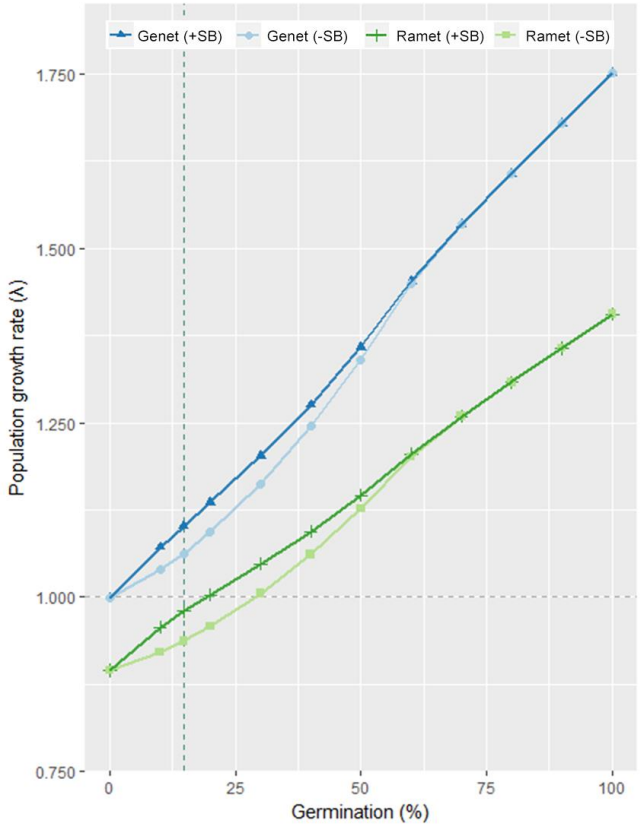
	152 rosettes (inc. 19 side-rosettes) and 14 seedlings	+ seed bank	- seed bank
BY RAMET	**+ clonality (6.7 %)**	0.9797	0.9375
	- clonality	0.9797	0.9375

Part 3 Population modelling – simulations

Clonality (0 – 100%)



Seed germination (0 – 100%)



- λ increases with increasing clonality and germination, except for the ramet models where clonality has no effect.
- Inclusion of a seed bank (SB) increases λ when there are dormant seeds.
- Importance of context-driven modelling.

$\lambda > 1$ **Population increasing**

$\lambda = 1$ **Stable**

$\lambda < 1$ **Population decreasing**

Conclusions and future directions

- Relatively low level of clonality (under 10 %) was detected at the study site. A large range of clonality exists in *Plantago* (plots ranged from 0 to 91 %).
- A soil seed bank was present but limited.
- Clonality and seed bank will matter to different extents at different sites and we would like be able to predict levels. PlantPopNet add-on protocols have been developed.

- How will *Plantago* be affected by climate change?
- It is anticipated that the PlantPopNet population models will allow us to understand plant populations in different environments and provide decision-making tools to effectively manage our ecosystems and natural resources.

Plant Pop Net

www.plantpopnet.com

Acknowledgements

THE UNIVERSITY OF SYDNEY

Glenda Wardle
Desert Ecology Research Group

Australian Network for Plant Conservation Inc

Plant Pop Net

Yvonne Buckley
Annabel Smith

Trinity College The University of Dublin

The Royal Botanic Garden Sydney

Maurizio Rossetto
Hannah McPherson

The Australian Botanic Garden Mount Annan

Cathy Offord
Jordan Scott
Lotte von Richer

This research was supported by Wardle Lab Funds and a University of Sydney Honours Scholarship.