

Measuring and managing genetic erosion in plant reintroductions:

Lessons from 20 years of genetic monitoring of the recovery of *Grevillea scapigera* (Proteaceae)

Siegy Krauss

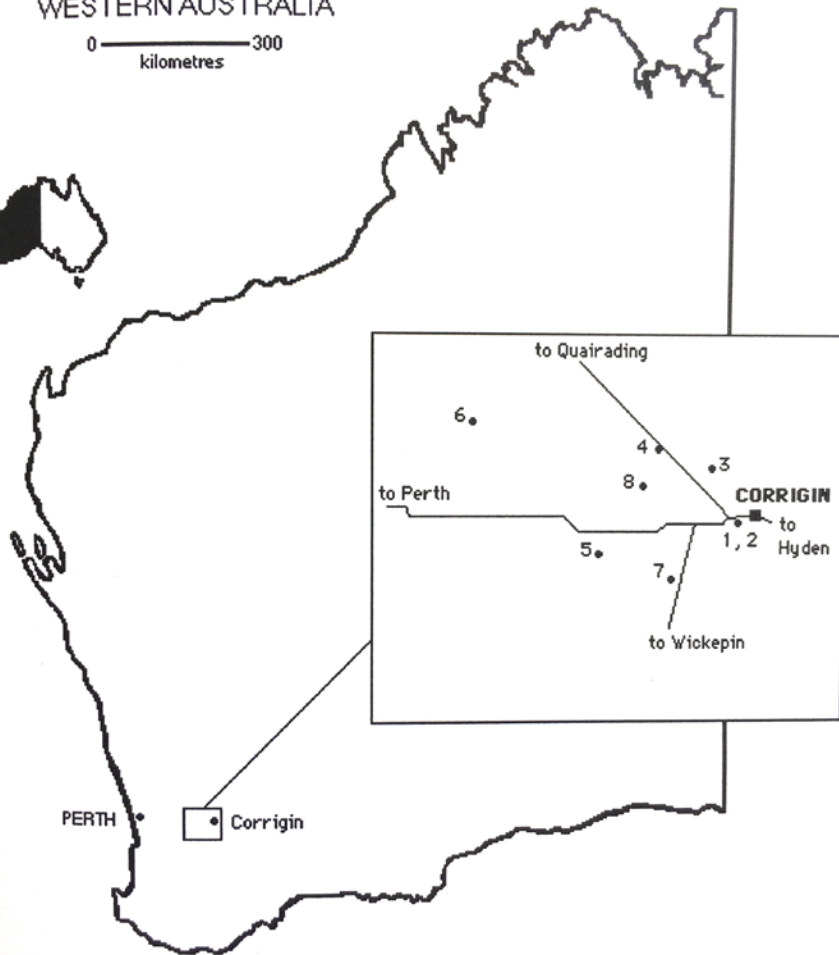
Bob Dixon, Bronwyn Ayre, Janet Anthony



First Collected: 1954
Presumed Extinct: 1984
Rediscovered in Wild: 1990

WESTERN AUSTRALIA

0 300
kilometres



Rossetto et al. (1995) Mol. Ecol
Rossetto et al. (1995) PhD



Translocation of *Grevillea scapigera*

1. Genetic assessment (RAPDs)
of 47 wild plants

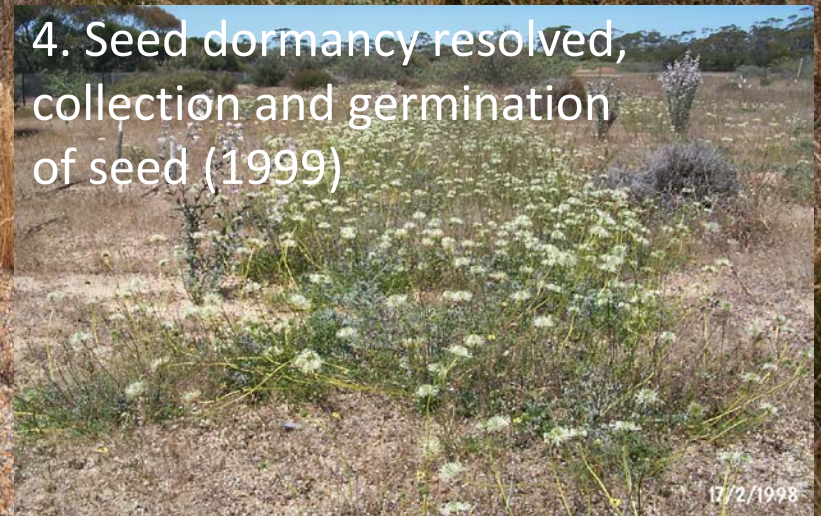
Rossetto et al 1995



3. Establishment (1996-1998)
>300 plants

2. Micropropagation of 10 clones
– seed germination impossible

4. Seed dormancy resolved,
collection and germination
of seed (1999)



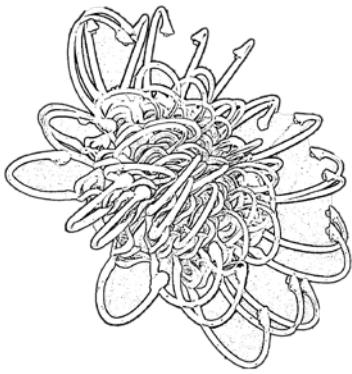


Initial Genetic Assessment of Founders and their F1 offspring (in 1999)

1. Assess genetic fidelity of the founding clones ($n = 10$) through *ex-situ* propagation
 2. Assess genetic erosion by comparing genetic variation between founders and offspring ($n = 103$)
 3. Assign paternity to progeny to assess reproductive success of each clone
- 143 AFLP markers

Krauss et al (2002) *Conservation Biology* 16:986-994.

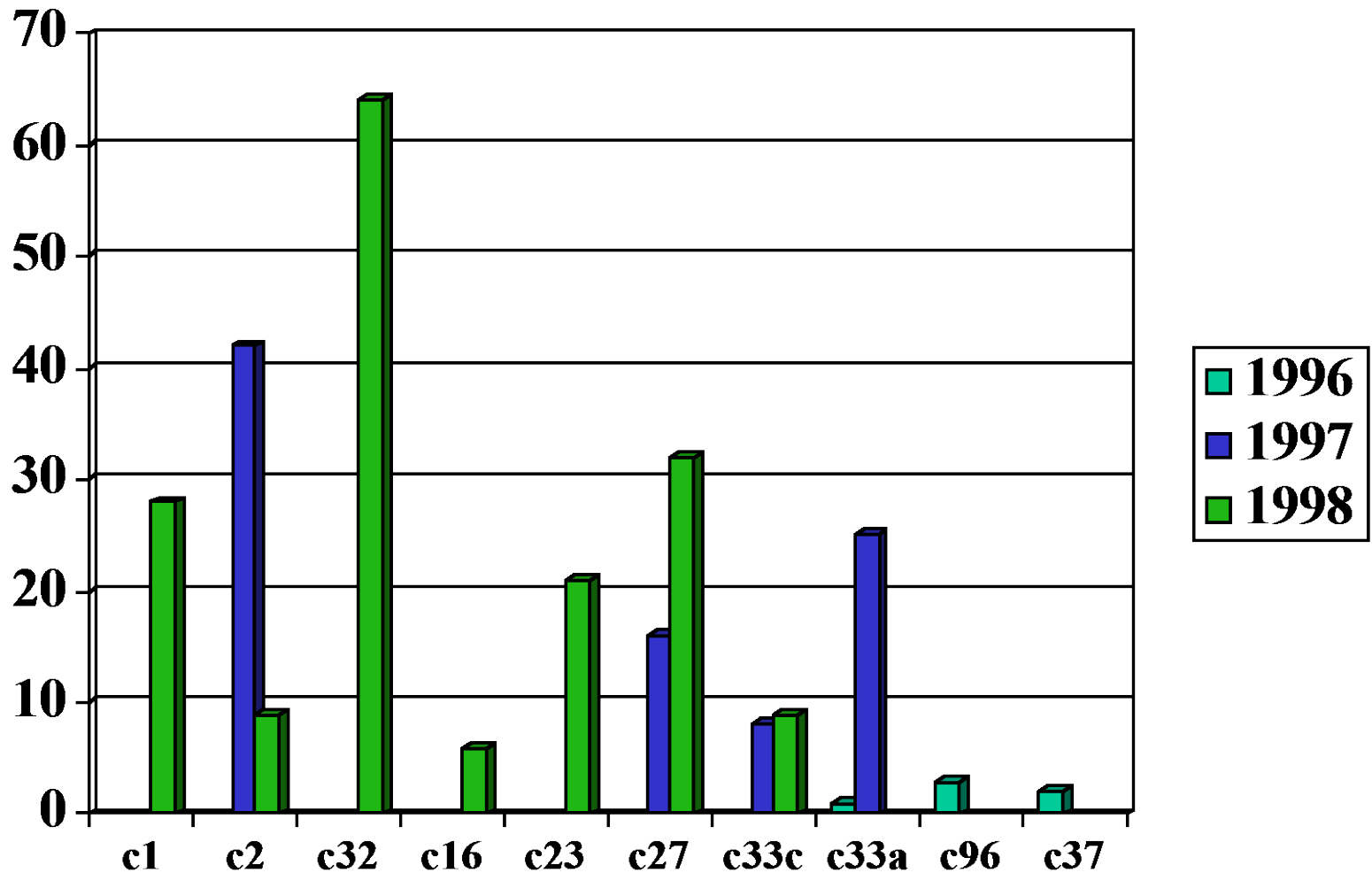




1. Genetic fidelity

of plants of each clone surviving in the Corrigin translocation site
in Jan 1999, by year of planting

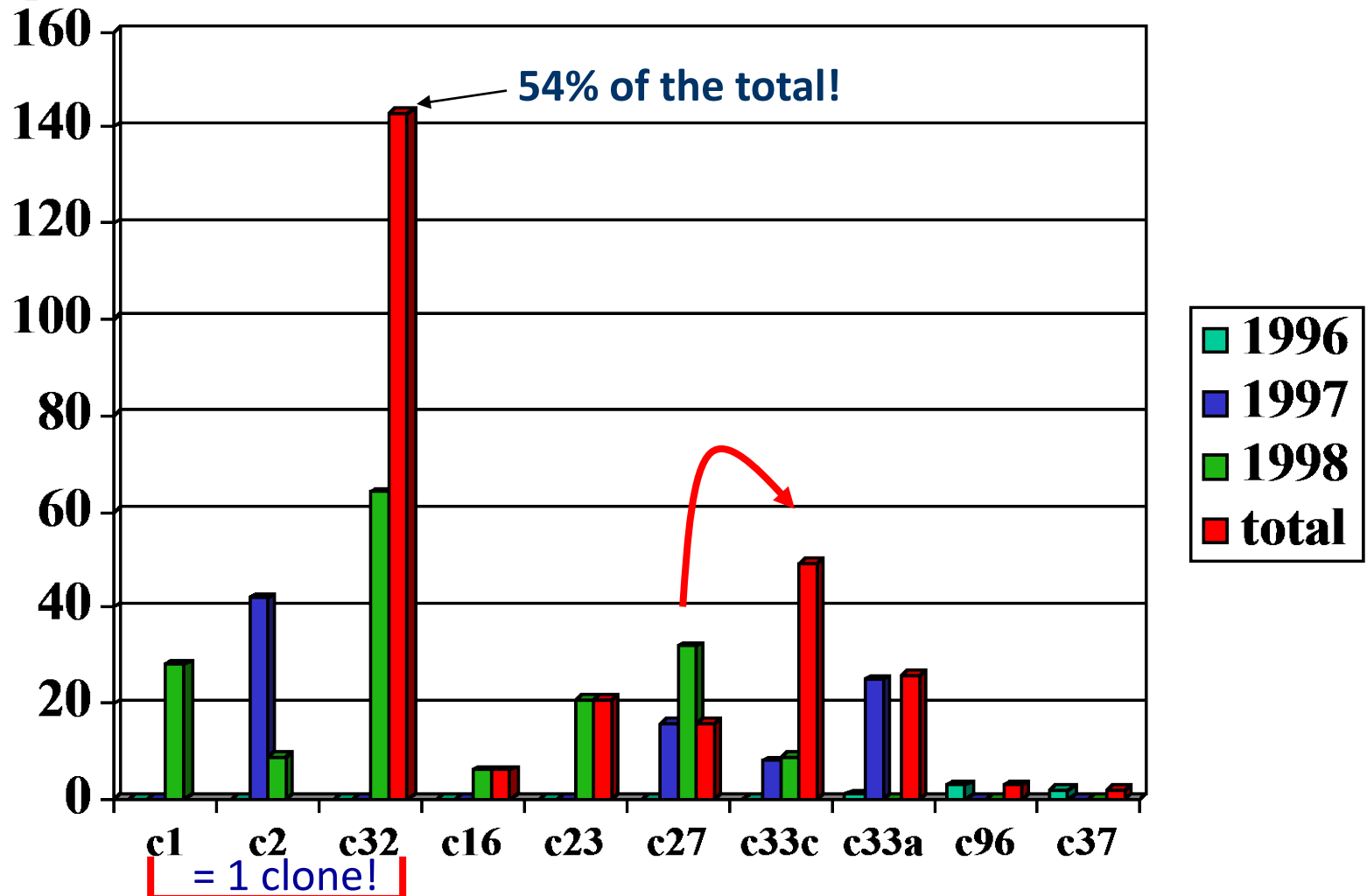
Total = 260



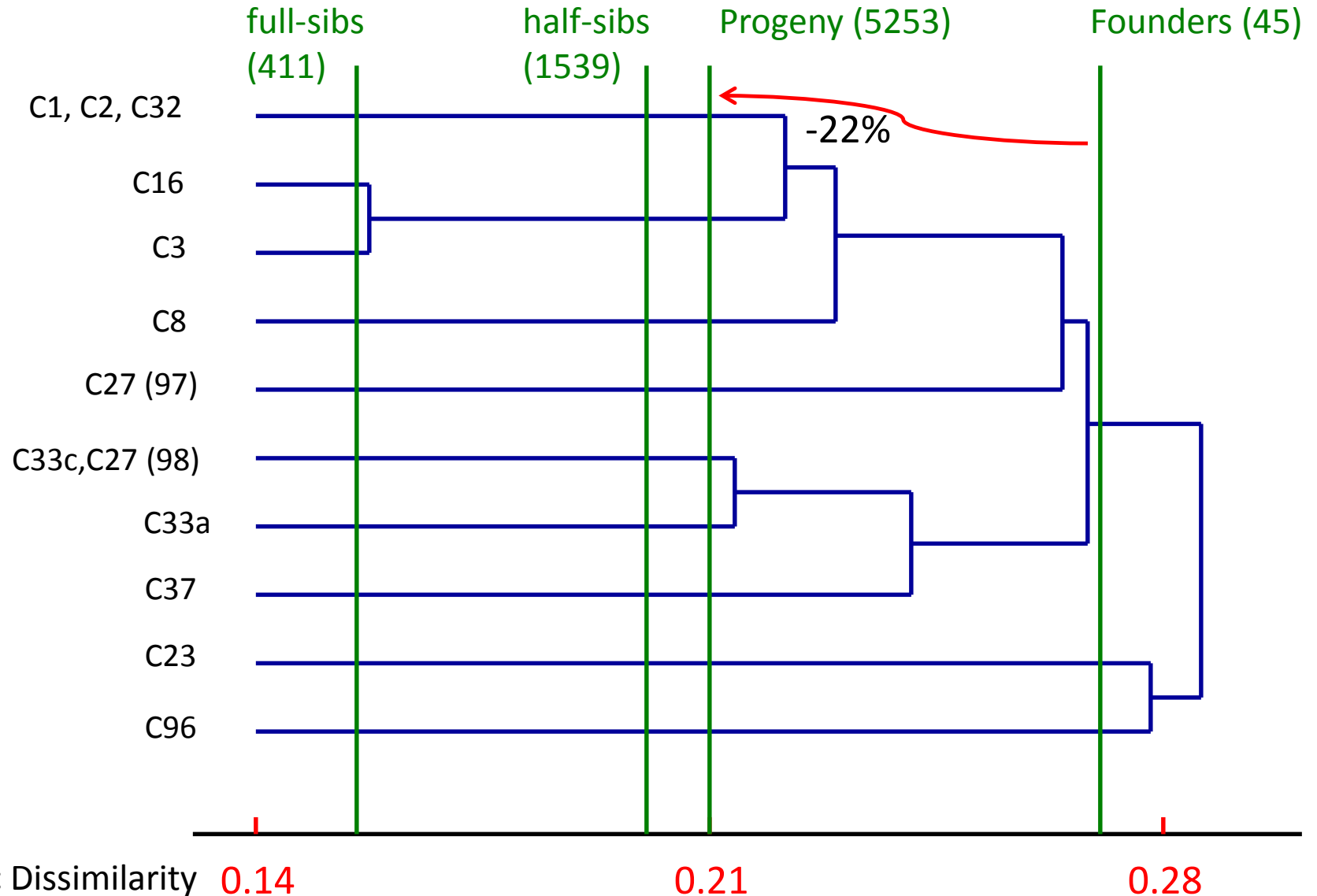


1. Genetic fidelity

actual #'s of each clone surviving



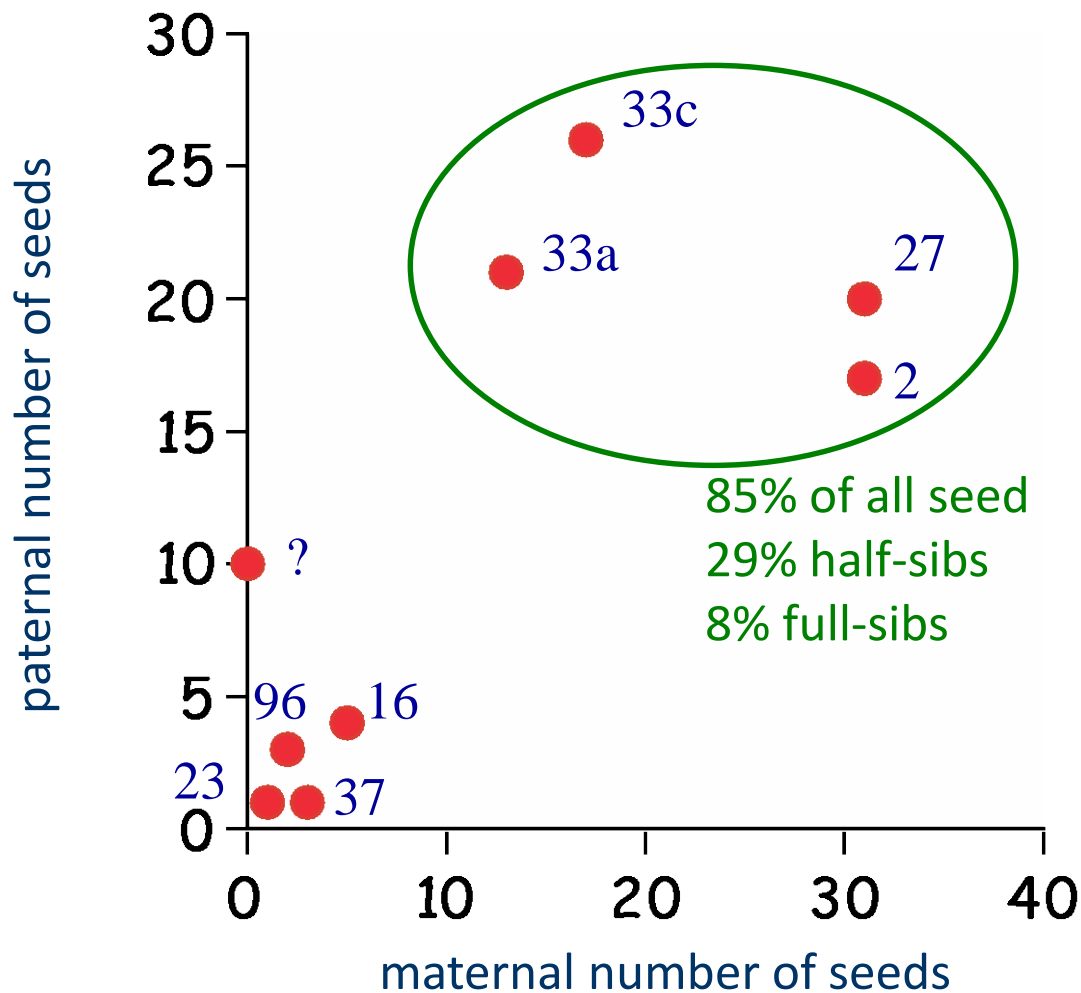
Average pairwise genetic dissimilarity at 143 AFLP markers –
1998 founders (n=10) and their F1 progeny (n=103) in 2000



3. Paternity assignment to assess reproductive success

103 Offspring: 102 outcross, 1 self

Maternal and paternal success of each founding clone



Effective population size (N_e) of translocated population in 1999

While $n = 260$:

$$1. N_e = H_0 / 2 (H_0 - H_1)$$

= **2.5**

$$2. N_e = (N v_k - 1) / [V_k - 1 + \text{var}(k) / V_k]$$

= **1.5**

where V_k = ave # of progeny per individual with variance $\text{var}(k)$



Strategies to reverse/prevent genetic erosion in translocations

- equalize founder numbers
- avoid population genetic structure in translocations

To avoid selfing, maximise multiple siring and minimise mean kinship of offspring

- increase population size

Add new wild clones as discovered (only source of new alleles)

Use seed, but this creates a size/kinship conflict.

- restore historical processes

Naturally small, ephemeral populations, so restore metapopulation dynamics with multiple pops and genetic connectivity through seed.

- Promote large seed banks and natural recruitment

Natural selection against inbred seed from large, long-lived seed bank

(*Ex-situ* propagation reduces selection against inbred seed).

Grevillea scapigera recovery time-line: a brief history

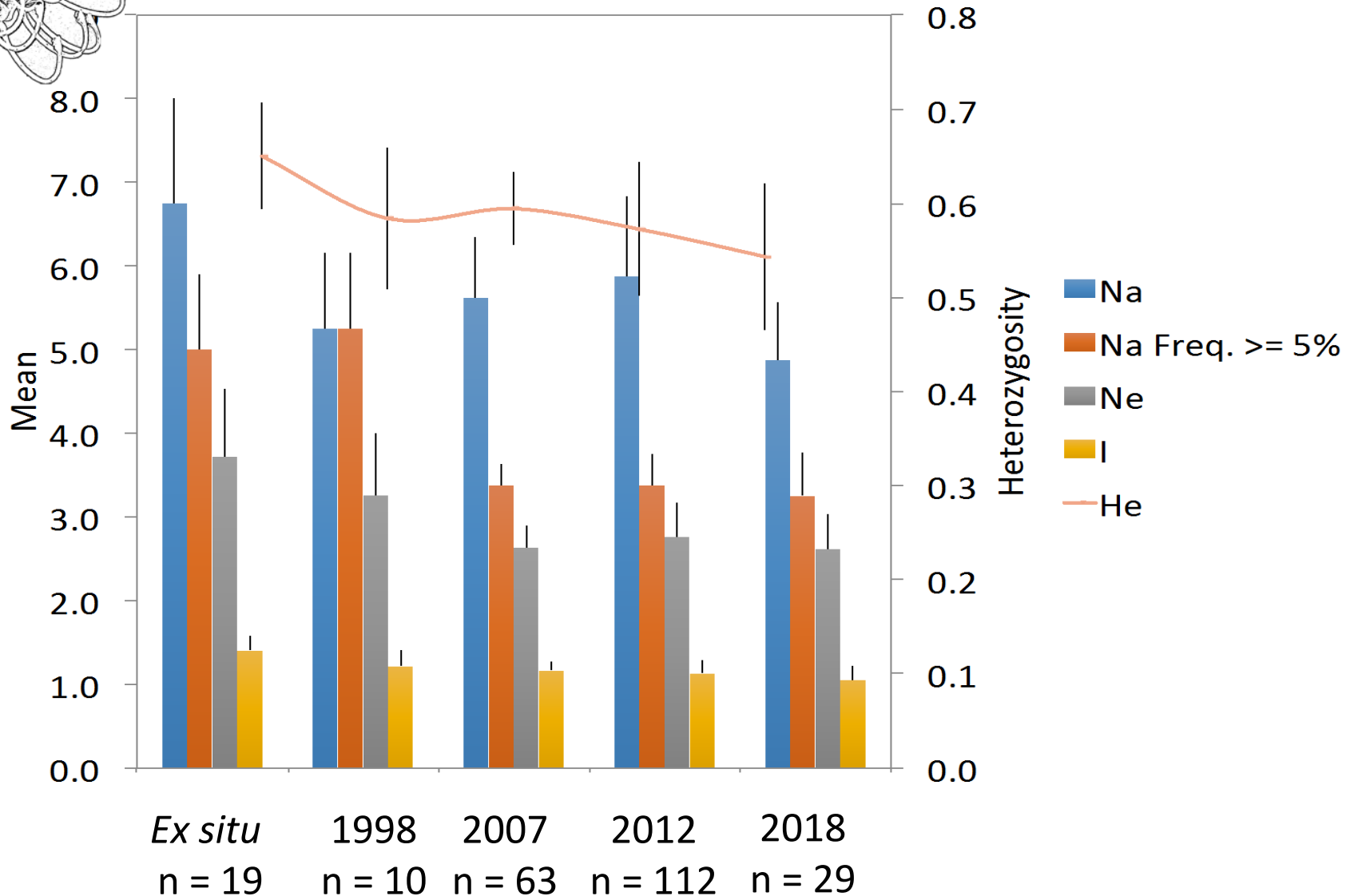
1954	<ul style="list-style-type: none">• First collection
1986	<ul style="list-style-type: none">• Presumed extinct when only known plant dies.
1987-1992	<ul style="list-style-type: none">• <i>Ex situ</i> collection (TC & cryo) established as new wild plants discovered
1994	<ul style="list-style-type: none">• 47 plants from 7 wild populations genotyped with RAPD (Rossetto <i>et al.</i> 1995)
1996-1998	<ul style="list-style-type: none">• First transplantation sites established, n = 260 in 1998, from tissue cultured plants of 10 representative clones, as deep seed dormancy prevents germination. All plants irrigated.
1999	<ul style="list-style-type: none">• Seed dormancy broken, germination possible.• Prolific flowering and seed set, >30,000 seed collected
2000	<ul style="list-style-type: none">• Founders and their offspring genotyped (AFLP), $N_e = 2$ (Krauss <i>et al.</i> 2002)
2000-2007	<ul style="list-style-type: none">• Seedlings propagated at Kings Park from wild pollinated seed sourced from, and planted back into, 3 translocation sites.• Supplemented with under-represented clones from <i>ex-situ</i> collection.
2004-2006	<ul style="list-style-type: none">• Experimental <i>in-situ</i> seed germination promoted.
2003-2011-	<ul style="list-style-type: none">• Estimated seed production > 1,000,000, into soil-stored seed bank p/a.• Natural recruitment in translocation sites: e.g. Corrigin 145 (2011), 147 (2014)
2012	<ul style="list-style-type: none">• 9 new wild collections incorporated into <i>ex situ</i> collection, n = 19.
2013/2018	<ul style="list-style-type: none">• Genetic assessment with microsatellites.

Genetic assessment of *G. scapigera* translocation F2 and F3 offspring

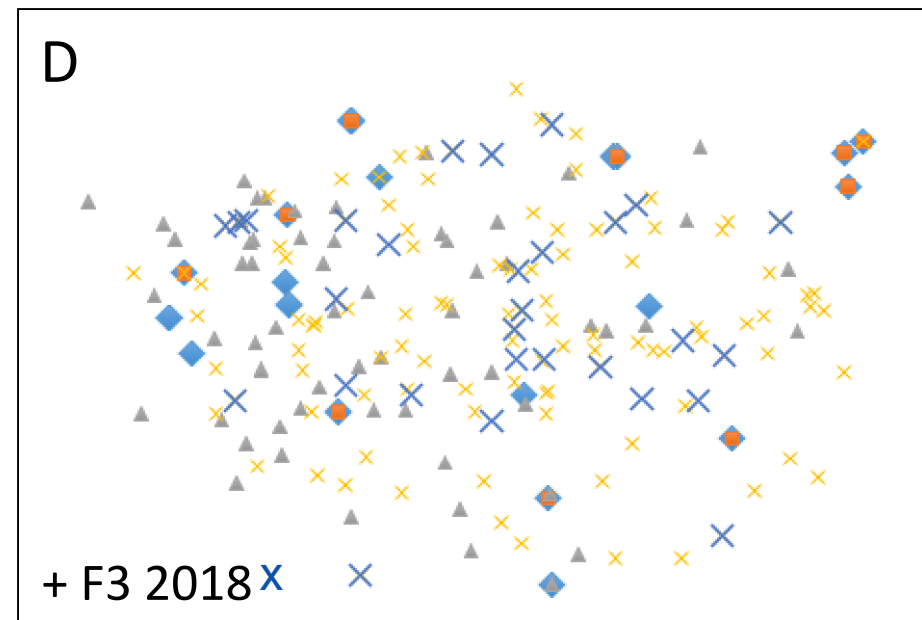
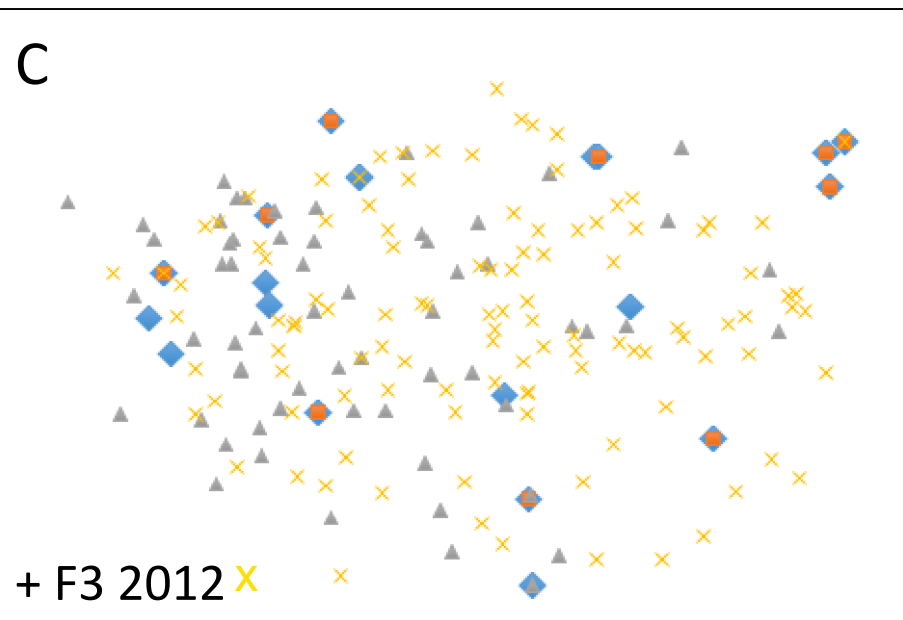
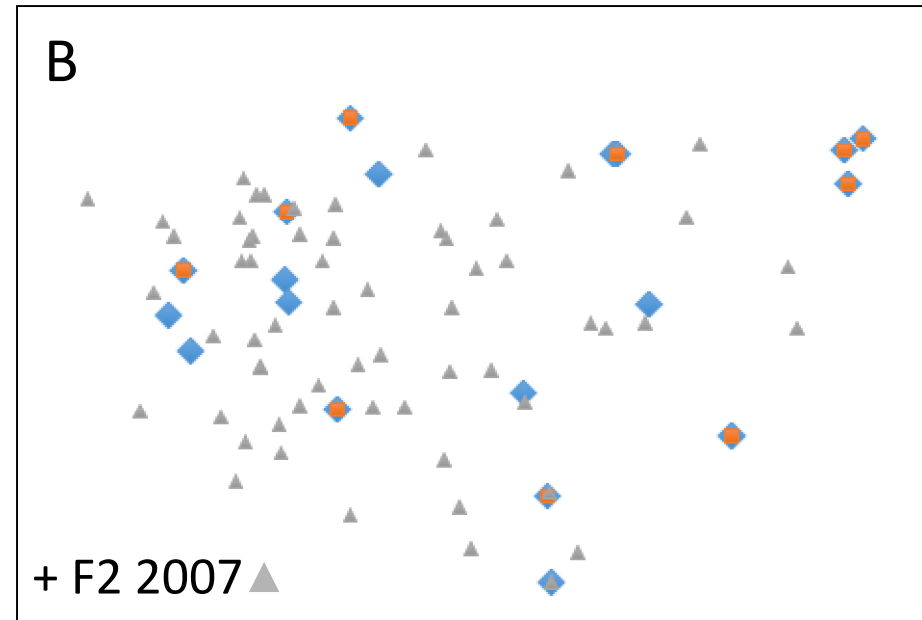
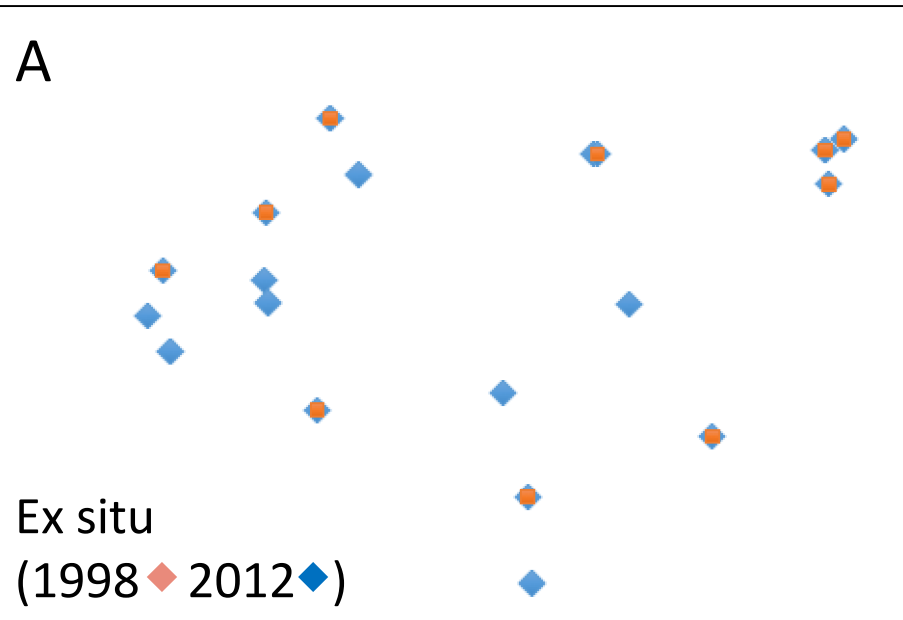
1. Assess genetic variation and fidelity of the 2012 *ex situ* collection
($N = 19$ MLGs from 24 accessions)
2. Assess genetic erosion by comparing genetic variation between
F0 (1998; $n = 10$), F2 (2007; $n = 63$), and F3 (2012/2018; $n = 112/29$)
3. Assess inbreeding depression



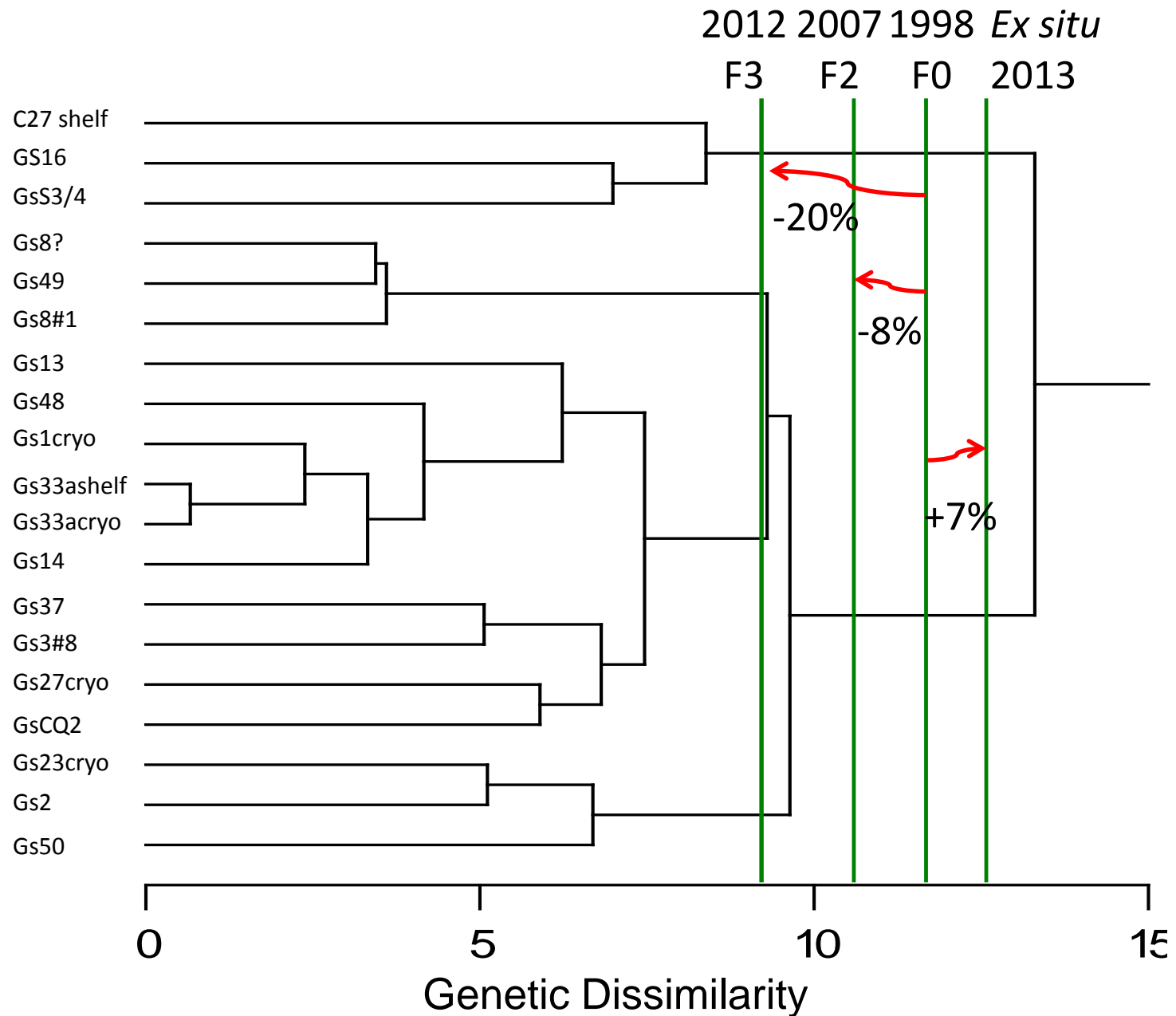
Microsatellite allelic diversity in *G. scapigera*: 9 loci
ex situ collection 2012,
translocation: 1998 (F0), 2007 (F2), 2012 (F3), 2018 (F3)



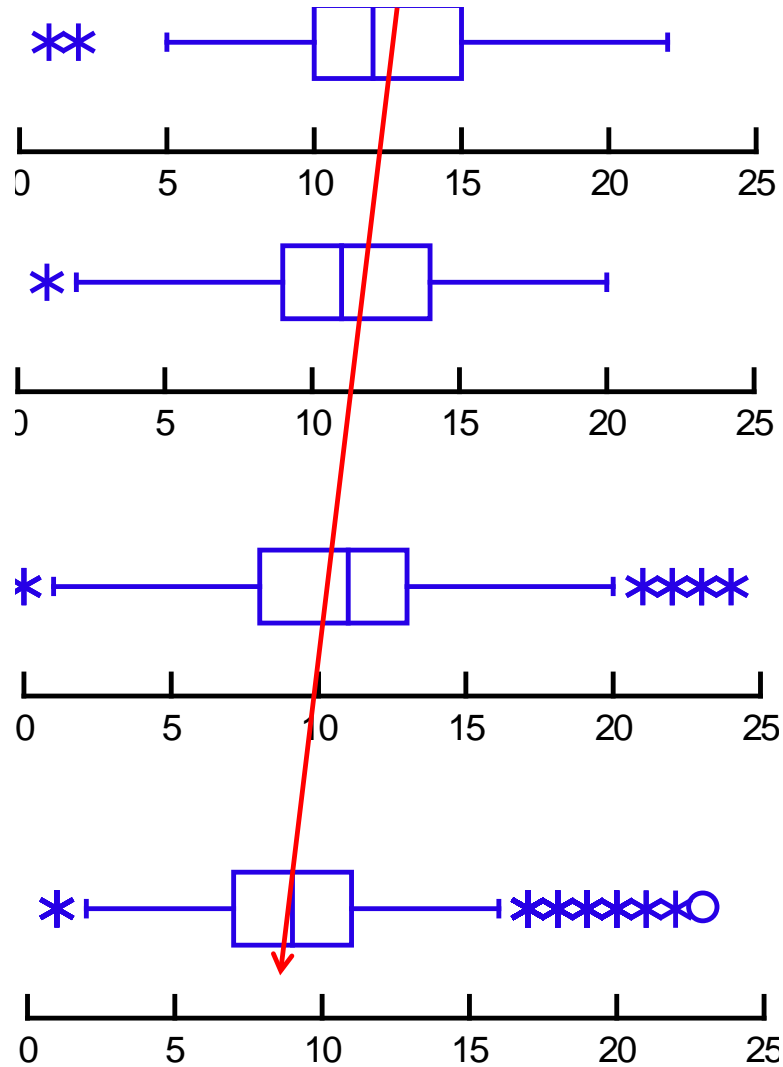
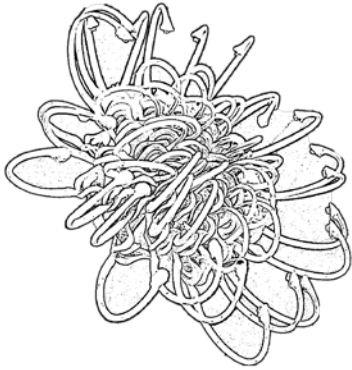
Ordination of genetic variation in *ex situ* collection and translocation site (1998-2018)



Average Pairwise Genetic Dissimilarity at 9 microsatellite loci



20% decline in mean pairwise Genetic Dissimilarity in 3 generations



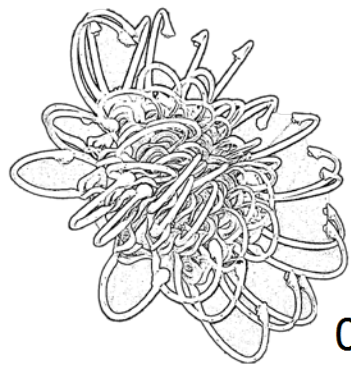
Ex situ

1998

2007

2012

Evidence for heterozygote advantage in natural recruits surviving at *G. scapigera* translocation site in 2018



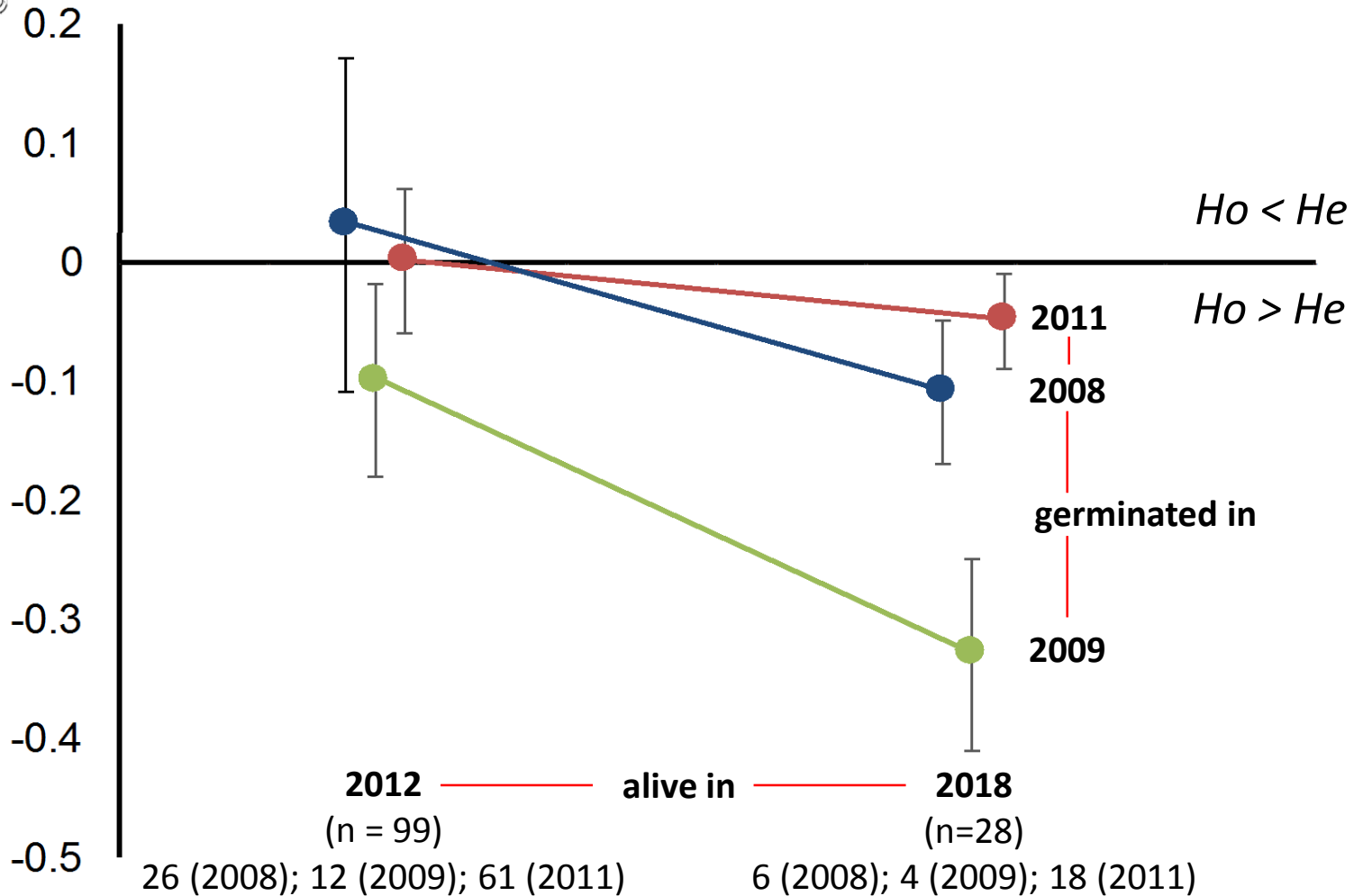
inbred



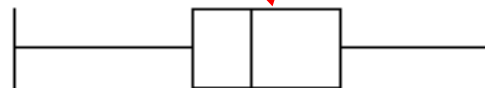
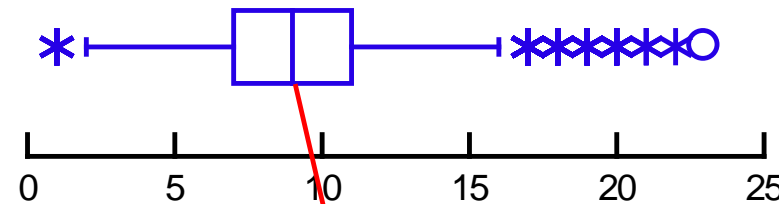
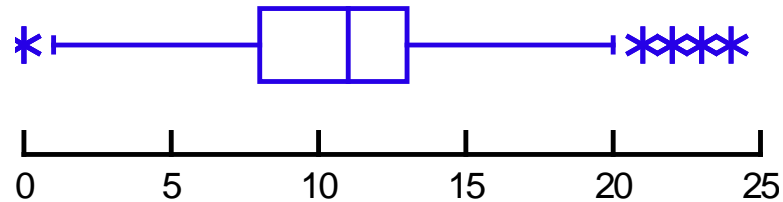
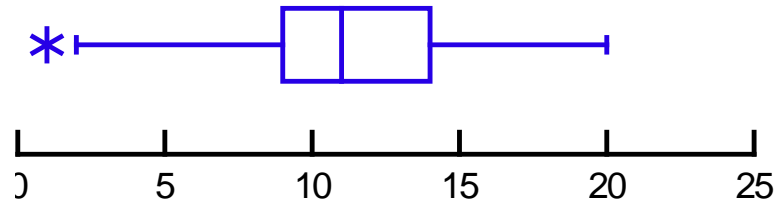
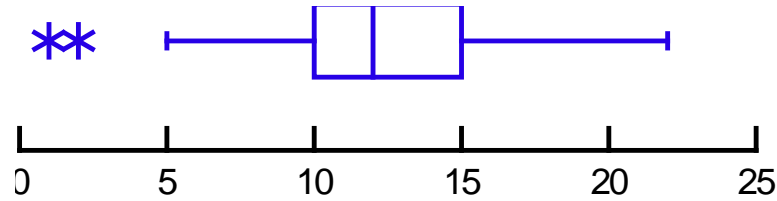
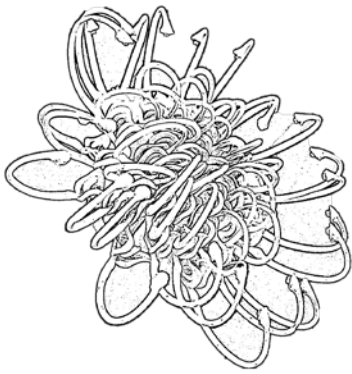
Inbreeding
coefficient

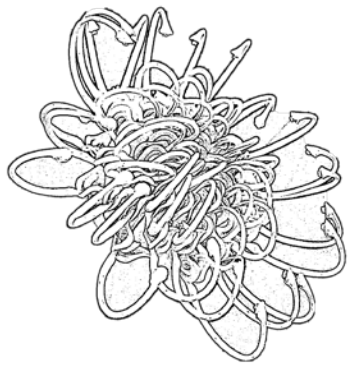


outbred



Increase in Pairwise Genetic Dissimilarity in 2018 survivors





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Conclusions:

- Genetic erosion stabilised post-2007.
- Pairwise genetic dissimilarity declined (1998-2012), rebounded with heterozygote advantage (2012-2018).
- Although mean dissimilarity decreased, range increased – (e.g. 23% of 2012 cohort more outbred than the mean for 1998).
- Mass flowering + pollinator services = very high seed set (>1M/year).
- Therefore, a lot of outbred seed to survive the inbreeding depression filter.
Thresholds?
 - Benefits to be gained from targeting relatively outbred individuals – promote wide outcrossing

Keys to successful translocation of *Grevillea scapigera*

- a 30-year journey
- mass flowering of hundreds of plants in multiple sites
- abundant insect pollinators
- mass seed set
- long-lived high-viability seed bank
- natural recruitment
- genetics managed for high diversity
- multiple secure sites = metapopulation
- floristically diverse, fenced, weed-free sites
- apparently now self sustaining
- *ex situ* collections (seed, tissue culture, cryo)
- underpinned by research
- dedicated volunteers

Acknowledgements:

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Grevillea scapigera recovery team + an army of dedicated volunteers