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Conservation genetics and ecology of two rare grevillea species

Tanya M. Llorens
University of Wollongong

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Conservation genetics and ecology of two rare *Grevillea* species

A thesis submitted in fulfilment of the requirements for the award of the degree

DOCTOR OF PHILOSOPHY

from the

UNIVERSITY OF WOLLONGONG

by

Tanya M. Llorens B. Sc. (Hons)

DEPARTMENT OF BIOLOGICAL SCIENCES

2004

Certification

I, Tanya M. Llorens, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Tanya Llorens

10 September 2004

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Abbreviations and conventions

NSW – The state of New South Wales, Australia

NPWS – The NSW National Parks and Wildlife Service

ROTAP – Rare or Threatened Australian Plants (Briggs and Leigh 1996)

AFLP – Amplified Fragment Length Polymorphism

RAPD – Randomly Amplified Polymorphic DNA

PCR – Polymerase Chain Reaction

All study sites and populations are referred to by a two- or three-letter abbreviation. Their full names are not given, both to facilitate ease of reading, and due to the sensitivity of information on populations of threatened plants.

Frequent reference is made in this thesis to studies with *Grevillea macleayana*. This species was previously known as *G. barklyana* ssp. *macleayana*, and the older name appears in some of the publications cited here.

Throughout this thesis, various genera and species from the family Proteaceae are often referred to. These genera include:

Banksia

Grevillea

Hakea

Lambertia

Leucadendron

Leucospermum

Persoonia

Telopea

Triunia

Leucadendron and *Leucospermum* are South African. The remaining genera are endemic to Australia, or, in the case of *Grevillea* and *Banksia*, 99% of their species are endemic to Australia (Harden 2002).

Abstract

Small and isolated plant populations have a higher probability of extinction – they are more susceptible to a range of environmental, demographic and genetic processes that may reduce population viability. In recent times, the number of populations and species that are susceptible to these processes has increased as a result of world-wide, human-induced habitat fragmentation. Habitat fragmentation typically reduces the formerly more continuous natural distribution of a species to a series of smaller and more isolated populations that occur in smaller and more isolated habitat patches. Such populations are often exposed to a range of additional processes that may threaten their viability, such as changes to disturbance regimes, environmental conditions and interactions with other species. However, our current understanding of the complex effects and interactions of these processes is poor. Species responses vary widely, studies are biased towards trees, herbs and self-incompatible species, and most studies investigate only one or two processes that may affect viability. Consequently, we are unable to make accurate predictions about the likely impacts of habitat fragmentation on population and species viability.

I tested several hypotheses about the impact of habitat fragmentation, small population size, and population isolation on populations of two *Grevillea* species (Proteaceae) that occur in the Sydney region of New South Wales, Australia. These species provided an opportunity to investigate some of the ecological and genetic consequences of small population size and isolation, and to contrast them between a species for which the small size and isolation of its populations is the natural state (*G. longifolia*) and one for which it arose recently due to severe habitat fragmentation (*G. caleyi*). This comparative approach is important in identifying the processes involved in reducing population and species viability. The species share many aspects of their biology and ecology. Both are large, perennial shrubs that are self-compatible and naturally bird-pollinated. They are fire-sensitive and regenerate post-fire by mass germination from a long-lived, soil-stored seed bank. Both species consist of populations that vary dramatically in size and degree of isolation.

I used microsatellite and AFLP markers to investigate aspects of the population genetics and mating system of these species, with the primary focus on *G. caleyi*. Both species showed a surprisingly large amount of genetic structuring among populations, although *G. caleyi* populations showed more structuring ($F_{ST} = 0.46$) than those of *G. longifolia* ($F_{ST} = 0.33$), despite being distributed over a much smaller area. In addition, for *G. caleyi*, most (63%) of the structuring was due to differences among recently-fragmented populations. By examining fine-

scale genetic structure within existing large populations, I determined that this was probably due to historic genetic structuring within formerly larger, more continuous populations. This has probably arisen due to both a lack of gene flow (no seed dispersal and limited pollen dispersal) and a large amount of inbreeding. Indeed, adult fixation indices were very high in *G. caleyi* (average $f = 0.40$, $f > 0$ in 16/18 populations). For both species, genetic diversity was not strongly correlated with population size. Genetic diversity was significantly lower in more isolated populations of *G. caleyi*, but this was probably due to a historic lack of gene flow to the more isolated parts of the species' natural range, rather than to recent fragmentation.

Levels of inbreeding (fixation indices) among adult plants did not vary with population size or isolation for either species. However, by genotyping fresh seeds from a range of small and large *G. caleyi* populations, I revealed that current outcrossing rates were much lower in small populations ($t = 0.18$ cf. 0.37). Observations of pollinator foraging indicated that this might be due to a very low visitation rate by birds and by a less diverse suite of species, resulting in a higher proportion of self-fertilisation. In contrast, even very small *G. longifolia* populations received many bird visits. In addition, *G. caleyi* plants in small populations were much smaller, had higher mortality, and produced fewer inflorescences and fruits, while this pattern was not apparent among *G. longifolia* populations. The contrast among the species in pollinator visits, plant vigour and reproduction may have been due to edge effects combined with the habitat degradation that was apparent at sites containing small *G. caleyi* populations. Small populations were typically found within very small and disturbed bush remnants, while small *G. longifolia* populations all occurred in relatively pristine habitat. Therefore, habitat quality rather than population size *per se* may be the most important factor that determines the mating system, plant vigour and fecundity in *G. caleyi*.

The lack of obvious impacts of habitat fragmentation on the genetic characteristics of adult *G. caleyi* plants may have been due to the soil-stored seed bank, which can contain seeds produced by at least two adult generations. Various authors have hypothesised that a persistent seed bank has the potential to reduce the rate of genetic change in a population. The seed banks of both *G. caleyi* and *G. longifolia* do appear to have this ability. I found that the seed bank of each species maintains the genetic characteristics of populations and stores genetic diversity and alleles that were not expressed in the extant adult plants. Nevertheless, the seed bank also showed greater spatial structuring than adults, which indicates that genetic changes may be occurring within these small populations despite the buffering power of the seed bank.

Finally, I investigated some aspects of the ecology of *G. caleyi* and *G. longifolia* seed banks, with the aim of increasing our understanding of this important conservation resource. Soil

sieving revealed that the seeds of both species occur at very low densities beneath adult plants ($1 - 6 \text{ m}^{-2}$), were vastly outnumbered by seed fragments, and were not found away from adult canopies. This supports previous evidence that indicated a lack of seed dispersal and very high rates of post-dispersal seed predation, which will restrict population size and extent. To some degree, the seed bank may buffer demographic changes that affected the previous adult generation – monitoring of post-fire seedling emergence revealed that population size typically increased, often dramatically, after a fire. Germination experiments showed that smoke elicited the greatest germination response from intact seeds of both species, and that dormancy polymorphism in the seed bank may allow both species to survive two fires in rapid succession and long inter-fire intervals. However, germination was low in field fire experiments, which may have been due to low fire intensity, and hence smoke production, resulting from a winter prescription burn. For both species, therefore, population viability may be compromised if the imposed fire regime includes fires that are too cool or too frequent.

This study has demonstrated, for these species, that small populations that exist in recently fragmented habitat patches are far more likely to experience adverse ecological and genetic effects than those in continuous, relatively undisturbed, bushland. The process of demographic and genetic decline in small *G. caleyi* populations is likely to continue with the ongoing pressures of edge effects, habitat degradation and pollinator declines, and the increased isolation of some populations. The seed bank may buffer these declines to some extent, but this ability is limited by a lack of habitat for population expansion, which means that effective population sizes will remain very small or decrease further. The various differences detected between small and large *G. caleyi* populations emphasises the importance of large populations, and the ecological processes occurring within larger habitat patches, for the long-term conservation of the species.

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