

Landscape genetics and sociobiology of Gould's long-eared bat (*Nyctophilus gouldi*) and the lesser long-eared bat (*N. geoffroyi*) in fragmented populations of south-eastern Australia

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The distribution of 14 *N. geoffroyi* and *N. gouldi* study sites across Victoria and South Australia. *Nyctophilus* were sampled in native vegetation (light grey) embedded within a matrix of hardwood (mid grey) and softwood plantations (dark grey) and agricultural land (white). The location of three *N. geoffroyi* roosts in farm buildings is displayed in the lower expansion showing their proximity to Warreanga NFR.

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Results of spatial autocorrelations performed in GENALEX illustrating mean  $r$  (—) with 95% upper and lower confidence levels (.....). Distance classes are displayed along the  $x$ -axis in km. Figures represent: (a) *N. gouldi* males, (b) *N. gouldi* females, (c) *N. geoffroyi* males, and (d) *N. geoffroyi* females.

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### **Table 3.2**

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Identification of dispersal events in GENECLASS determined with a significance level of  $p < 0.05$ . The inferred source population and the population in which an individual was trapped are displayed. Fourteen sampled populations are defined: Nan = Nangwarry, War = Warreanga, Dry = Dry Creek, Hon = Honans, Ann = Annya, Wee = Weecurra, Otw = Otways, Gra = Grampians, MtN = Mt Napier, MtE = Mt Eccles, Hot = Hotpur, Str = Strathdownie.

**Table 4.3**

Summary of population genetic measures and sample numbers across 14 *N. geoffroyi* populations. N = number of samples,  $A_R$  = allelic richness,  $A_P$  = private alleles,  $H_O$  = observed heterozygosity,  $H_E$  = expected heterozygosity, SH = standardised heterozygosity, IR = internal relatedness,  $F_{IS}$  = the inbreeding coefficient.

**Table 4.4**

Pairs of relatives identified using KINGROUP. Four types of relationships were examined: parent-offspring, full siblings, half siblings and cousins. Results are presented for 14 populations across south-eastern South Australia and western Victoria. Relationships were established with a confidence level of  $p < 0.05$ .

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# Thesis Abstract

Habitat fragmentation represents one of the greatest threats to biodiversity, yet for the second largest mammalian order Chiroptera we have only just begun to assess the impacts of this threatening process on population connectivity and genetic diversity. Many aspects of chiropteran ecology remain unknown due to their cryptic lifestyle and difficulties in applying traditional observational and field-based techniques. At the time of this PhD project's conception there were no published studies utilising genetic techniques to address the influence of habitat fragmentation on any chiropteran species. Since that time two studies have been published, in 2009 and 2011. I add to this new body of literature by conducting genetic analyses to assess population connectivity and genetic diversity in two congeneric vespertilionids, *Nyctophilus gouldi* and *N. geoffroyi*. The study was conducted in western Victoria and south-eastern South Australia across a landscape comprising continuous and fragmented regions of native habitat. Populations within continuous forest provided a benchmark for parameters including gene flow, genetic diversity and social structure, for comparison with forest fragments. This thesis also capitalises on the underutilised potential of molecular techniques for the study of chiropterans. I applied molecular approaches to assess dispersal strategies and social structure in both species offering novel ecological insights. Four data chapters covering these topics are outlined below.

Chapter 2 describes the isolation and characterisation of 16 microsatellite markers developed to facilitate this research. I utilised next generation sequencing technology (454) to generate a microsatellite DNA library and employed Multiplex Ready Technology (MRT) as a flexible and cost effective method to test primers and design marker panels for screening. DNA was isolated from *N. gouldi* resulting in 15 loci, while cross amplification in *N. geoffroyi* produced 7 reliable loci.

Chapter 3 addresses the impact of habitat fragmentation on the forest and woodland specialist *N. gouldi*, which is listed as endangered in South Australia. Based on roosting requirements, rarity in the agricultural landscape and limited dispersal ability I predicted that *N. gouldi* populations would display reduced gene flow and signs of isolation as a result of habitat fragmentation. This prediction was confirmed by my analyses which identified reduced population connectivity, decreased genetic diversity, elevated measures of relatedness and

inbreeding, and altered demography within fragmented populations isolated by  $\geq 27$  km of agricultural land. Agricultural distances  $< 2$  km did not influence population connectivity providing a benchmark for habitat restoration to improve connectivity and mitigate population isolation in this species. Management recommendations include the enhancement of population connectivity between threatened SA populations, and recognition of a unique Management Unit at the Grampians National Park.

The fourth chapter investigates the influence of habitat fragmentation on *N. geoffroyi* for comparison with *N. gouldi*. In contrast to *N. gouldi*, *N. geoffroyi* is a habitat generalist that occupies a diverse range of ecosystems and which is commonly recorded within agricultural landscapes. *N. geoffroyi*'s presence in modified habitat coupled with plastic ecology and roosting requirements led to the prediction that the species would display limited impacts from habitat fragmentation. My analyses again confirmed this prediction with *N. geoffroyi* displaying virtually no response to habitat fragmentation and a panmictic population structure across the study region. The comparison between *N. geoffroyi* and *N. gouldi* provided an opportunity to test the merit of several proposed predictors of bat vulnerability to habitat fragmentation, in particular wing morphology, matrix tolerance, specialisation and geographic range. The much touted predictor wing morphology failed to predict differing responses from the two species while the following three predictors listed above received further support from this study. I conclude that wing morphology may still be a useful predictor of bat vulnerability to habitat fragmentation when coupled with other indicators such as matrix tolerance and habitat specialisation.

The fifth and final data chapter utilises molecular analyses to assess several previously unknown aspects of *N. gouldi* and *N. geoffroyi* ecology, dispersal strategies, mating systems and social structure. *N. gouldi* displayed patterns consistent with female natal philopatry, male biased dispersal and a polygynous mating system, while no such evidence was found for *N. geoffroyi*. Results for *N. geoffroyi* may have been influenced by larger population sizes which, coupled with higher dispersal rates, may have masked any evidence of sex-biased dispersal. Both species displayed significant numbers of relatives at the population level, with *N. gouldi* displaying particularly high levels of related females. *N. geoffroyi* displayed higher numbers of relatives at the roost level indicating that kin selection may play an important role in social structure and cooperative roosting. Despite significant numbers of related *N. geoffroyi* at the roost level, the vast majority of pairwise comparisons indicated no

relationship between individuals suggesting that the dominant driver of sociality and cooperative behaviour may not be solely based on relatedness. Nevertheless, high incidence of related females at the population level for *N. gouldi*, and at the roost level for *N. geoffroyi*, suggests that the bonds between related females are an important aspect of *Nyctophilus* behavioural ecology and social structure.

## Statement of Authorship

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Nicholas Fuller and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

This thesis represents an original and independent piece of work. All significant aspects of the work were conducted by myself including field work, laboratory work, data analysis and interpretation, the production of manuscripts and the procurement of ethics approval, research permits and all funding accrued to facilitate this project. My supervisors Steven J. B. Cooper and Susan M. Carthew contributed to the production of manuscripts and provided supervisory support and guidance. S. Cooper provided additional guidance with data analysis and interpretation.

GIS maps displayed as Figures 3.1, 4.1 and 5.1 were produced by Christopher J. Medlin and the cover image, a photograph of *N. gouldi*, was taken by Terry Reardon.

This thesis is presented as a series of manuscripts with Chapters 2-5 intended for publication in peer-reviewed journals co-authored by myself, and my supervisors Steven J. B. Cooper and Susan M. Carthew.

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Nicholas C. Fuller