Species conservation in fragmented landscapes: Implications for the Grey-crowned Babbler

Kate P. Stevens

B.Env.Sc. (Hons.) Deakin University

A thesis submitted in total fulfilment of the requirements for the Degree of

Doctor of Philosophy

29 December 2015

Life and Environmental Sciences

Deakin University

Australia
DEAKIN UNIVERSITY
ACCESS TO THESIS - A

I am the author of the thesis entitled

Species conservation in fragmented landscapes:
Implications for the Grey-crowned Babbler

submitted for the degree of

Doctor of Philosophy

This thesis may be made available for consultation, loan and limited copying in accordance with the Copyright Act 1968.

“I certify that I am the student named below and that the information provided in the form is correct”

Full Name: Kate Stevens
Signed: [Signature Redacted by Library]
Date: 29 December 2015
DEAKIN UNIVERSITY
CANDIDATE DECLARATION

I certify that the thesis entitled

Species conservation in fragmented landscapes:
Implications for the Grey-crowned Babbler

submitted for the degree of

Doctor of Philosophy

is the result of my own work, and where reference is made to the work of others,
that due acknowledgment is given.

I also certify that any material in the thesis which has been accepted for a degree or
diploma by any university or institution is identified in the text.

Full Name:  Kate Stevens
Signed:  
Date:  29 December 2015
I dedicate this work to the continuance of biodiversity on Planet Earth, but most particularly, to an especially quirky, fun-loving, fascinating and enchanting bird species, the Grey-crowned Babbler. [Amongst others], it is they who taught me about science, perseverance, commitment and what it is to truly grow up in the Australian Bush.

Grey-crowned Babbler (*Pomatostomus temporalis*)
“There is no such thing as only one right answer to a question, and I have never yet heard an answer that is completely wrong and no use at all.”

Dr AR Pepper
31/12/39 – 05/10/11
Preface

This thesis is a compilation of my own work. I designed the methods for the study, with guidance from my three supervisors: Raylene Cooke (principal), Andrew F. Bennett (research) and Rohan Clarke (research). I conducted all of the fieldwork and collected all samples used in this research. I was guided in the genetic laboratory work by Fiona Hogan, and in the technical genetic components of the thesis by Fiona Hogan and Katherine Harrisson. I also conducted all of the statistical and data analysis presented with initial guidance from Greg Holland, Katherine Harrisson and Nevil Amos. I drafted, revised and was the corresponding author for the second manuscript that is in press for publication (Raylene Cooke was corresponding author on the first published manuscript). I took all of the photographs included in this thesis.

All of the thesis chapters have been written as manuscripts for publication, and hence reflect the collaborative nature of the works by using the plural form of the first-person ‘we’. Each chapter is also self-contained and some repetition occurs, particularly in the methods sections. All references have been placed at the end of the thesis not at the conclusion of each chapter. Chapter 2 has been published (PLoS One), Chapter 3 is currently in press (Emu), and Chapters 4 and 5 are in preparation for publication. These manuscripts have been co-authored with the above mentioned supervisory and collaborative panel and they have therefore contributed to the ideas presented in each.
The thesis publications are as follows:

**Chapter 2:**

**Chapter 3:**

**Chapter 4:**
Stevens K, Harrisson KA, Hogan FE, Cooke R and Clarke RH (in prep). Gene flow declines for a cooperatively-breeding bird reflect over two centuries of landscape-scale habitat loss and fragmentation. Evolutionary Applications

**Chapter 5:**
Acknowledgements

This thesis would not have been possible without the dedication and support from a number of key people. Firstly my supervisors whom consistently provided me with the drive to keep going: Raylene Cooke, Andrew F. Bennett and Rohan Clarke. Raylene, your guidance, support and encouragement has been second to none. Thank you for your constant availability even while constructing your own brood, practical advice, sharing your own experiences and your professionalism and friendship. I am truly grateful. D2, thank you for taking on this project, it was made all the better and more enjoyable with your expert guidance and humour. Thanks also for sharing your bird knowledge, providing (and explaining) constructive criticism, true concern for my occasional field work dilemmas and having faith in me to finish. Andrew, your wisdom, advice, strategic questioning, quiet support and assured guidance has been invaluable. Your dedication to the project and my burgeoning status of novice researcher has been encouraging. Additional to my supervisors, but certainly not secondary to them, is my infinite gratitude to Fiona Hogan, Katherine ‘Starfish’ Harrisson, Greg Holland and Nev Amos. Their collaboration and strategic guidance, wise council and encouragement was priceless. I feel blessed that these people particularly, are part of this thesis journey.

I pay homage to other enthusiastic and dedicated researchers of the Grey-crowned Babbler who seemed so happy to help with knowledge, time and even some field work: Doug Robinson, Chris Tzaros and Nigel Lacey for your advice and data sets.
Doug and Nigel, your enthusiasm and personal knowledge of these birds were inspirational forerunners to my own. Chris, I am forever indebted to your mysterious suggestion to study a bird called a Grey-crowned Babbler. What a journey you started! Caroline Blackmore who offered advice and kind encouragement to go forth and study GCBs. Ian ‘Davo’ Davidson for keeping me updated with current local GCB research and sharing knowledge. The (always) cheery staff and fellow PhDs at Deakin University. In particular I would like to thank Clorinda Schofield and Jess Bywater for dealing with all of the technical and financial aspects of the project, and kept me laughing. Marian Weaving, who has been my constant cheerful, tearful and forgiving companion throughout our entire university journey together: Mazdazopolous, you kept me going when it felt ridiculously impossible.

Funding for this project was most generously provided, by: the Holsworth Wildlife Research Endowment, the Birds Australia Stuart Leslie Bird Research Fund, Birds Australia Professor Allan Keast, Jill Landsdowne Trust Fund, Deakin University, and my generous and supportive friend, Lois Gurney. Without such support, the field and genetic components particularly would have been unachievable. I shall be pleased to remain forever in their debt.

I must thank the generous assistance of my dedicated volunteers whose practical help made the PhD journey rich with laughter and wondrous experience and without who, this thesis would not have achieved all that it has. Moya, Greg, and Kep Smith who kept me in good company and superb accommodation. Nigel Lacey enthusiastically
accompanied my field work trips in the south-east, and happily continues to discuss all things Babbler with me. Naoko Takeuchi who’s unmatched organisational skills, joy and dedication to conserving birds worldwide needs to earn her a Nobel Prize. Thank you also to John Miller, Lisa Moore, Julie King, Tammy Davies, Chris Connelly, Al Heffron, Dave I.

My family, thanks is barely enough. Particularly, I would like to give recognition to my Mum and Dad, and their constant support and encouragement throughout my studies. Mum, you make the most beautiful bird bags in the world and Parsey, you are my inspiration more often than you can imagine. Bruce who came in when the fun part was over and supported me through the toughest parts with wonderings, love and an engagement ring!

As always, my deepest gratitude to my friends (of) BW.
Table of Contents

Preface v
Acknowledgements vii
Abstract xx

1 Introduction 1

1.1 The decline of the world’s species 2
1.2 A quality problem 3
1.3 Environmental change affecting biological processes 4
1.4 Woodland birds in south-eastern Australia 6
1.5 The Grey-crowned Babbler 8
1.6 The theme and question focus of this thesis 11

2 What determines habitat quality for a declining woodland bird in a fragmented environment: The Grey-crowned Babbler Pomatostomus temporalis in south-eastern Australia? 15

2.1 Abstract 16
2.2 Introduction 17
2.3 Methods 19

2.3.1 Study species 19
2.3.2 Study area 20
2.3.3 Site selection 22
2.3.4 Bird surveys 23
2.3.5 Vegetation surveys 24
2.3.6 Regional habitat comparisons 24
2.3.7 Variable selection for modelling 25
2.3.8 Model development and selection 26

2.4 Results 29
2.4.1 Comparison of habitat attributes within and between regions 29
2.4.2 Breeding success (fledgling presence/absence) 30
2.4.3 Group size 34

2.5 Discussion 35

3 Genetic structure and sex-biased dispersal of a declining cooperative-breeder, the
Grey-crowned Babbler (*Pomatostomus temporalis*), at the southern edge
of the species’ range.

3.1 Abstract 43
3.2 Introduction 44
3.3 Methods 48
3.3.1 Study area, site selection and DNA sampling 48
3.3.2 Molecular methods 50
3.3.3 Microsatellite descriptive statistics 51
3.3.4 Genetic diversity 52
3.3.5 Population genetic structure 52
3.3.6 Isolation-by-distance and dispersal patterns 53

3.4 Results 54
3.4.1 Landscape structural differences 54
3.4.2 Microsatellite loci statistics 55
3.4.3 Genetic diversity across regions 55
3.4.4 Population genetic substructure amongst regions 56
3.4.5 Isolation-by-distance and dispersal patterns 59
3.5 Discussion 62

3.5.1 Influences of geographic distance and barriers on genetic diversity and population structure 62

3.5.2 Fine-scale population structure and sex-specific effects of habitat loss and fragmentation 65

3.6 Conclusions and management recommendations 66

4 Gene flow declines for a cooperatively-breeding bird reflect over two centuries of landscape-scale habitat loss and fragmentation. 70

4.1 Abstract 71

4.2 Introduction 72

4.2.1 Genetic implications for a species experiencing spatial and genetic population structure: The case of the Grey-crowned Babbler 77

4.3 Methods 78

4.3.1 Sampling 78

4.3.2 Molecular methods 79

4.3.3 Microsatellite performance, heterozygosity and genetic diversity 80

4.3.4 Contemporary gene flow among subpopulations 81

4.3.5 Long-term gene flow estimation 82

4.3.6 Temporal effective number of migrants per generation 83

4.3.7 Temporal effective population sizes 84

4.3.8 Modelling population history 85

4.3.9 Signature of bottlenecks within subpopulations 86

4.4 Results 87

4.4.1 Genetic diversity amongst subpopulations 87

4.4.2 Contemporary gene flow rates and ancestry 88

4.4.3 Long-term gene flow rates 90
4.4.4 Long-term and contemporary effective number of migrants per generation 90

4.4.5 Long-term and contemporary effective population sizes 93

4.4.6 Demographic history of subpopulations 94

4.4.7 Bottleneck signatures within subpopulations 94

4.5 Discussion 97

4.5.1 Gene flow decline despite evidence of long-distance dispersal 97

4.5.2 Signatures of genetic bottlenecks and small effective population sizes 98

4.5.3 Influences of drift and migration shaping contemporary population structure 99

4.6 Conclusion and recommendations 100

5 Modelling landscape-level structural and functional connectivity pathways for dispersal and gene flow of a declining cooperatively-breeding avian species. 102

5.1 Abstract 103

5.2 Introduction 104

5.2.1 Conserving the genetic viability of populations in large fragmented landscapes 104

5.2.2 Evidence of gene flow between declining species requiring genetic investigation 107

5.3 Methods 111

5.3.1 Sampling 111

5.3.2 Molecular methods 112

5.3.3 Microsatellite performance and pairwise individual genetic distance 113

5.3.4 Building a landscape for resistance modelling 113
5.3.5 Building landscape distance models

5.3.5.1 Null model surface
5.3.5.2 Surfaces based on tree cover
5.3.5.3 Surfaces based on landscape resistance heterogeneity
5.3.5.4 Surfaces based on nearest neighbours
5.3.5.5 Formulating distance matrices and current maps using Circuitscape

5.3.6 Assessing relationships between genetic and landscape distances using a reciprocal causal modelling framework

5.4 Results

5.4.1 Analyses of genetic data
5.4.2 Marginal Mantel correlations between genetic and landscape distances
  5.4.2.1 Isolation-by-distance
  5.4.2.2 Isolation-by-resistance based on binary landscapes
  5.4.2.3 Isolation-by-resistance based on landscape resistance heterogeneity

5.4.3 Partial mantel correlations between genetic distance and landscape distance
  5.4.3.1 Isolation-by-distance effects
  5.4.3.2 Isolation-by-resistance based on binary landscapes
  5.4.3.3 Isolation-by-resistance based on heterogeneous resistance landscapes

5.4.4 Original and reciprocal causal modelling
5.4.5 Circuitscape landscape current mapping

5.5 Discussion

5.5.1 Influences of structural connectivity on functional connectivity:
  insights into movement ecology of the Grey-crowned Babbler
5.6 Management implications 139

6 Conclusion and recommendations 141

6.1 Thesis overview 142

6.1.1 Summary of key findings 142

6.1.2 Ecological investigations 143

6.1.3 Biological investigations 149

6.2 Conservation implications of fragmented landscapes 150

6.3 Management of a declining species in fragmented habitat at a landscape-level 153

6.4 Future studies 157

References 160

Appendix 1 198

Appendix 2 202
List of Tables

Table 2.1 Explanatory variables for models of breeding success and group size for the Grey-crowned Babbler. 27
Table 2.2 Model-selection results for breeding success (fledgling presence/absence) and mean group size of the Grey-crowned Babbler. 31
Table 2.3 Authorship statement for Chapter 2 40
Table 3.1 Genetic diversity metric averages of the Grey-crowned Babbler within its southern edge-of-range population. 56
Table 3.2 Cluster assignment of Grey-crowned Babblers within the three study regions 57
Table 3.3 Authorship statement for Chapter 3. 69
Table 4.1 Genetic diversity of the Grey-crowned Babbler within six subpopulations at their southern range limit. 89
Table 4.2 Estimates of recent (previous 2-3 generations) mean gene flow rates per generation among six Grey-crowned Babbler subpopulations. 92
Table 4.3 First-generation migrants identified among six genetic subpopulations of the Grey-crowned Babbler in southern parts of its range. 93
Table 4.4 Estimates of long-term and contemporary effective number of migrants per generation and effective population sizes for two geographically separated regions of Grey-crowned Babbler in the southern part of their distribution. 94
Table 4.5 Three models showing Bottleneck signature values for Grey-crowned Babbler individuals from six subpopulations. 96
Table 5.1 Values used for landscape distance surfaces for developing each landscape mode 117
Table 5.2   Summary of causal modelling framework results for marginal and partial Mantel tests showing Mantel correlation ($r$) and $p$ values for Euclidean and resistance distances correlated with individual genetic distances of the Grey-crowned Babbler.  

Table 5.3   Reciprocal causal modelling r values estimated from partial Mantel $r$ differences between supported isolation-by-resistance models. 

Table 6.1   Overview of thesis themes and broad findings for the ecology and biology of the Grey-crowned Babbler *Pomatostomus temporalis*. 

Table A1.1  Group size of the Grey-crowned Babbler and the number of groups that recorded breeding success at least once during the period June 2010 to April 2011, for study sites across the west, south-east and north-east study regions. 

Table A1.2  Comparison between regions of habitat characteristics in Grey-crowned Babbler territories based on ANOSIM. 

Table A1.3  Within-region similarities in habitat characteristics of Grey-crowned Babbler territories. 

Table A2.1  Global characterisation of 13 microsatellite loci of the Grey-crowned Babbler within its southern-most distribution. 

Table A2.2  FRAGSTATS landscape dispersed and aggregated statistics for tree cover in the east and west study regions.
# List of Figures

| Figure 1.1 | Global range of the Grey-crowned Babbler depicting the range extent for *Pomatostomus temporalis rubeculus* and *P.t.temporalis*. 9 |
| Figure 2.1 | Study area in south-eastern Australia centred on three distinct regions: west (Kerang), south-east (Benalla), and north-east (Rutherglen). 21 |
| Figure 2.2 | Model-averaged coefficients and associated 95% confidence intervals for explanatory variables included in models of (a) breeding success (fledgling presence/absence); and (b) group size of the Grey-crowned Babbler. 32 |
| Figure 2.3 | Predicted probability of occurrence of Grey-crowned Babbler fledglings as a function of average group size. 33 |
| Figure 3.1 | The distribution of the Grey-crowned Babbler, study region and population genetic structure of *P.t.temporalis* in its southernmost range within the bounds of the study area. 58 |
| Figure 3.2 | Grey-crowned Babbler spatial genetic structure as a function of distance within the study population for (a) all sampled individuals; (b) males and females. 60 |
| Figure 4.1 | The global distribution of the Grey-crowned Babbler, and the study region and sample site locations of the subspecies, *P.t.temporalis* in its southernmost range. 76 |
| Figure 5.1 | Map of Australia indicating the location of the study area within Victoria, and site locations within the study area. 108 |
| Figure 5.2 | Current map based on isolation-by-distance and used to predict important connectivity areas between Grey-crowned Babbler subpopulations in southern areas of their range. 130 |
Figure 5.3  Current map based on resistance values of the ‘mosaic100NN’ resistance model and used to predict important connectivity areas between Grey-crowned Babbler subpopulations in southern areas of their range. 131

Figure 5.4  Current map based on resistance values of the ‘mosaic50’ resistance model and used to predict important connectivity areas between Grey-crowned Babbler subpopulations in southern areas of their range. 132

Figure 5.5  Current map based on resistance values of the ‘mosaic5’ resistance model and used to predict important connectivity areas between Grey-crowned Babbler subpopulations in southern areas of their range. 133

Figure A1.1  Non-metric multi-dimensional scaling ordination of study sites within three regions based on the differences in habitat attributes. 201
Abstract

Anthropogenic landscape modification typically results in habitat loss, fragmentation and degradation; processes recognised as major drivers of the recent, and continuing, declines of global biodiversity. There is an urgency to understand the implications of habitat loss and fragmentation for ecological processes, to ameliorate threats to species persistence in modified landscapes. The Grey-crowned Babbler *Pomatostomus temporalis* (Pomatostomidae), is a cooperatively-breeding woodland bird that is declining in the southern part of its range as a result of major habitat loss and fragmentation in agricultural landscapes. This thesis investigates habitat attributes, social organisation, population genetic structure and dispersal behaviour of the Grey-crowned Babbler at different spatial scales. The dual aims of this thesis are to determine the consequences of the threatening processes and contribute to effective conservation management of this vulnerable species.

The study included a large sample of 405 individual Grey-crowned Babblers from 72 family groups, and was undertaken over the period August 2010 to April 2012. The study area encompassed ~22,500 km² across north-central and north-eastern Victoria, Australia, stratified into three geographically distinct regions: west (centred on Kerang 36.12 °S, 143.72 °E), north-east (centred on Rutherglen 36.06 °S, 146.46 °E) and south-east (centred on Benalla 36.55 °S, 145.98 °E). These regions support the largest Grey-crowned Babbler populations in Victoria and represent the southern limit of the species’
current range.

I identified species’ territories within the three regions and recorded presence or absence of fledglings, and family group size, as surrogates of territory quality. These measures were modelled in relation to: habitat attributes within territories, extent of surrounding wooded vegetation, isolation from neighbouring groups and the size of the neighbourhood population. Fledgling presence showed a strong positive association with group size, but no other environmental variables predicted breeding success. Average family group size was larger in the west than in other regions, but after accounting for region no other environmental variables at either territory- or landscape-scale were important predictors of group size. The parameters I employed as biologically meaningful measures of habitat quality; group size and breeding success, did not provide evidence for differences in territory quality across the study regions. Relationships between group size and environmental variables may be obscured by longer-term trends in group size, which are not evident from a ‘static’ survey over short periods.

Genomic DNA was isolated from blood samples from 135 Grey-crowned Babblers originating from 40 groups across the three study regions. This was used to estimate genetic diversity, population substructure, local relatedness and dispersal patterns. Individuals showed high heterozygosity within regions, and differences of the number of private alleles between regions. This suggests that levels of connectivity for Grey-crowned Babblers vary between the three regions. Four distinct genetic clusters
revealed a population substructure that was consistent with treeless landscapes acting as strong barriers to gene flow. In contrast to previous studies, my data indicated a male-biased dispersal pattern and significant isolation-by-distance patterns for females at fine spatial scales.

I also used genetic methods to test whether contemporary levels of gene flow (i.e. the proportion of individuals that are migrants within a subpopulation) across the landscape are lower than historical levels, for six genetic subpopulations of the Grey-crowned Babbler. The contemporary (previous 2-3 generations) mean rates of gene flow among subpopulations were low (range: 0.01-0.19), while the mutation-scaled, long-term rate of mean gene flow for a subset of migratory routes were more moderate (range: 5.0-11.3). The contemporary effective number of migrants per generation for two stratified regions incorporating the six subpopulations, east and west, indicated a major decline in gene flow between the two regions over time. Estimates of contemporary effective population size between the east and west regions was 19.7 and 17.0 respectively; well below effective population sizes required for long-term population viability. Alternative models of demographic history indicated that genetic drift was a greater influence on all subpopulations than was gene flow, and five of the six subpopulations showed evidence of recent bottlenecks. The results are indicative of a substantial decrease in landscape-level structural connectivity which has affected the functional connectivity of the Grey-crowned Babbler in the study area.

To assess the contribution of landscape structural connectivity to genetic
connectivity between geographically separated subpopulations of the Grey-crowned Babbler, I employed a reciprocal causal modelling framework to compare alternative hypotheses of factors influencing gene flow i.e. isolation-by-distance (IBD), and isolation-by-resistance (IBR). Matrices of pairwise genetic distances were correlated both with isolation-by-distance and isolation-by-resistance matrices, as estimated with marginal Mantel and partial Mantel tests ($r$). The model that best explained the patterns of individuals’ genetic distances was identified as the hypothesis with the most support relative to all others. Marginal Mantel $r$ provided greater support for isolation-by-resistance models than isolation-by-distance. Partial Mantel $r$ supported two isolation-by-resistance models, based on assigning the highest resistance values to land lacking tree cover, for all birds and for males separately. The isolation-by-resistance model identified as the best fit for females incorporated high resistance values for areas lacking tree cover, for major roads and for areas at elevation levels >300 m (non-habitat). All marginal Mantel $r$ values were highest for models correlated with female genetic distances, indicating that landscape structure has a more pronounced effect on females than that observed for all birds or for males separately. Reciprocal partial Mantel $r$ for each of the three genetic distance data sets (i.e. all; male; female) were fully supported by only one isolation-by-resistance model out of a possible 12. Supporting models differed among the data sets. ‘Current maps’ produced from Circuitscape depicted riparian corridors and networks of roadside vegetation as likely critical pathways of landscape-level structural connectivity that may effectively enhance functional
connectivity of the Grey-crowned Babbler in the study area.

This study contributes to better understanding of the effects of habitat loss and fragmentation on a cooperatively-breeding, woodland-dependent species. In particular, it has identified the importance of connectivity and gene flow for populations of a threatened and declining species. This work forms a basis for conservation actions that will benefit conservation of the Grey-crowned Babbler. Such actions are also relevant to other sedentary and cooperatively-breeding bird species, as well as other woodland-dependent species occupying fragmented ecosystems.
1 Introduction

A Grey-crowned Babbler helper ~2+ year old. Helpers of this species delay dispersal to assist in rearing subsequent generations of (typically) their siblings, but will also emigrate to other groups to help rear unrelated offspring.
1.1 The decline of the world’s species

Large-scale anthropogenic modification of natural ecosystems has been relentless since global industrialization commenced in the 18th century (Vitousek et al. 1997; Fletcher 2008; Rockstrom et al. 2009). As a result, habitat loss, fragmentation and degradation in the world’s temperate forests, woodlands and grasslands for example, have been pervasive in both intensity and extent (Fahrig 1997). The relationship between habitat loss, fragmentation and global declines in biodiversity is well documented (Saccheri et al. 1998; Villard et al. 1999; Swift and Hannon 2010), highlighting that negative effects from habitat loss and fragmentation are many, and differ amongst species (Selwood et al. 2009; Harrisson et al. 2012; Cerame et al. 2014).

The negative effects from landscape modifications such as population decline and loss of landscape connectivity, often have interactive natures and hence, are likely to exacerbate the severity of the consequences for species and populations. When a loss of habitat such as diminishing patch size occurs, habitats can become more vulnerable to invasion from introduced plants and animals (Howes et al. 2014), further reducing resource availability both in terms of quantity and quality.

Many ecological processes such as dispersal and gene flow, rely on landscape connectivity for their occurrence (Sunnucks 2011). A decline in structural connectivity (the physical features and construct of a landscape) can often lead to reduced functional connectivity (the ability of a landscape to facilitate movement of organisms through it), as species movement through a landscape becomes more difficult and fraught with risk (Doerr et al. 2011). Habitat fragmentation then, may lead to negative cascades between
population processes as dispersal and gene flow becomes restricted for vulnerable species such as poor dispersers. Negative cascades from interacting ecological processes that increase rates of population decline ultimately affect species persistence in a landscape (Bruno et al. 2003; Ewers and Didham 2006; Hagen et al. 2012). Understanding the nature of these interactions therefore, is crucial for conservation management. Detecting the underlying processes of decline however, is difficult, because a number of ecological and environmental factors can influence population change. Some species may be more vulnerable to particular threats than others, serving to complicate investigations. For example, an increase in the edge-to-area ratio of forest or woodland vegetation resulting from habitat fragmentation can impact the diversity and/or richness of woodland-dependent species from an increased abundance of species that favour forest edges (Bennett et al. 2015). Such species may compete for resources such as nest sites and food, with species that depend on forest vegetation and result in a reduction in species richness of out-competed forest-dependent species. Furthermore, population sizes at broader scales may also suffer decline as other local patches succumb to the invading competitor (Bennett et al. 2014).

1.2 A quality problem

Habitat quality can be affected both directly and indirectly by fragmentation. Direct impacts are often readily visible and include disturbance processes such as invasive species of plants and/or animals (Porlier et al. 2009; Dickson et al. 2013), and agricultural intensification, such as cropping regimes and high use of chemical sprays
Indirect impacts on habitat quality can often go unseen, nevertheless consequences are no less severe than direct effects. Roads for instance, affect habitat quality both directly and indirectly. The construction of a road through a forested area immediately reduces total tree cover of the forest and increases the risk of invasive exotic species. Roads can directly change dust and light levels in the area immediately surrounding it, as well as indirectly impacting soil water content, surface water availability and run-off behaviour, thereby affecting habitat quality for some species hundreds of meters from the road (Trombulak and Frissell 2000).

Isolated habitat patches are more susceptible to various disturbance processes than continuous habitat. Isolated patches are more likely to have an increased edge-to-area ratio and hence the buffering effects associated with habitat extent is diminished. Patch edges facilitate the introduction of exotic plants and can be causal factors in the degradation of the understory and ground substrates within patches (Franken and Hik 2004; Uezu and Metzger 2011). Consequently, there is a pressing need to understand the causal mechanisms driving the current epidemic of biodiversity decline and to identify how differences in habitat quality affect species of concern. Indicators of habitat quality that are derived from an assessment of habitat use across a species’ range is one area of research that has merit. This is especially so if findings are transferable to a variety of taxa in similar areas or circumstances.

**1.3 Environmental change affecting biological processes**

A decline in habitat quality often has detrimental effects on an individual’s fitness and therefore the fitness of their offspring (Pidgeon et al. 2006). Degraded habitat
typically leads to a decline in resource availability. A lack of critical resources in turn, increases the need for individuals to disperse to areas that are more conducive to offspring production and which also support patch colonisation. Small, isolated patches are also more likely to have lower habitat quality than large patches, and species persistence in the landscape is reliant on the ability to move through landscapes as established territories becomes saturated (Sirami et al. 2009). Habitat fragmentation however, has the capacity to directly impede an individual’s ability to move with relative safety, owing to greater distances between patches. Ramifications of increasing dispersal distances exacerbate these risks further and can affect species at local-, regional-, and landscape-levels.

Impeding individual movements across a landscape by increasing distances to available territories and/or to non-related individuals can lead to the isolation of populations. The creation of a number of smaller isolated populations can be detrimental to the viability of a species, making it more susceptible to disease, stochastic events and climate change, due to the potential loss of genetic diversity as a result of genetic drift (Méndez et al. 2014). The ongoing development in molecular methods, spatial and statistical analyses and computational power in recent years, has provided the means to examine the effect habitat fragmentation has on animal movements in an emerging field which is ‘landscape genetics’. Recent landscape genetic studies have used a variety of analytical and statistical methods to investigate the effects of fragmentation on such fundamental processes as mating systems, gene flow, landscape connectivity and contemporary and historical migratory levels (Amos et al. 2012; Barr et
Australian landscapes have undergone rapid and extensive modifications during the last 250 years since colonisation from Europe in the 18th Century (Australian Surveying and Land Information Group 1990; McAlpine et al. 2007). Land clearing for agricultural production, resource extraction, urbanisation and development of infrastructure (e.g. roads) have resulted in broad-scale changes to native vegetation. In many regions, these processes have reduced functionally connected ecosystems to landscape mosaics of human production lands peppered with disparate and degraded habitat patches (Bradshaw 2012). The impacts on natural ecosystems have been profound, resulting in the decline of numerous species, some to the point of extinction (Ford et al. 2009; Szabo et al. 2011). Anthropogenic landscape modifications continue to affect the persistence of many species (Environment Australia 2011). A combination of habitat loss, fragmentation and degradation of habitats is implicated in the decline of over 80% of threatened birds in Australia. Amongst species most affected by these processes are those that rely on connected habitat at a landscape-level, habitat specialists and restricted dispersers (Ford et al. 2001).

There has been much research on avian species in fragmented landscapes in Australia (Saunders 1989; Major et al. 2001; Lindenmayer et al. 2002; Watson et al. 2005; Skroblin et al. 2014). Such studies have shown that habitat fragmentation can result in varying effects on different avian species (Ford et al. 2001), as has been found globally (McGarigal and McComb 1995; van den Berg et al. 2001; Fuhlendorf et al. 2002;
Coulon et al. 2010). This includes disrupted dispersal, inbreeding, genetic bottlenecks and population declines (e.g. Red-tailed Black Cockatoo (*Calyptorhynchus banksia*), Maron 2005; Golden-shouldered Parrot (*Psephotus chrysopterygius*), Garnett and Crowley 1997; Brown Treecreeper (*Climacteris picumnus*), Doerr et al. 2011; Superb Fairy-wren (*Malurus cyaneus*), Harrisson et al. 2013; Grey Shrike-thrush (*Colluricincla harmonica*), Pavlova et al. 2012). The loss of multiple species of birds in south-eastern woodlands and forests is driven by a complexity of interacting factors (Ford et al. 2001), including reduced food availability, reduced nesting resources, changes to habitat quality and increased competition for these reduced resources.

Recent work has investigated multiple species to understand specific patterns of decline in regions of south-eastern Australia (Haslem and Bennett 2008; Harrisson et al. 2012; Harrisson et al. 2014). Such studies have described different species responding differently to habitat loss and fragmentation. Landscapes reaching critical thresholds of decline in habitat cover (<10%) for instance, result in a disproportionate reduction in species richness (Radford et al. 2005). Species such as the Superb Fairy-wren were found to tolerate landscape fragmentation at a landscape-scale, but population processes at a fine-scale were adversely affected e.g. gene flow (Harrisson et al. 2013).

Three Honeyeater species in the same landscape also displayed different effects of species decline and were either tolerant to any loss of structural connectivity or had disappeared from landscapes with <17% tree cover remaining (Harrisson et al. 2014). Amos et al. (2012) used molecular approaches to describe the impacts of landscape fragmentation on the genetic structure of multiple avian species in south-eastern
Australia also. Amos et al. (2014) found that several sedentary species were more likely to experience increasing levels of genetic isolation from increasing distances rather than isolation from landscape resistance. Conversely, species that were more mobile or more resilient to fragmentation effects were more likely to experience isolation-by-resistance (i.e. movement costs associated with differing resistance levels across surfaces) and suffered little to no effects on their population genetic structure (Amos et al. 2014).

1.5 The Grey-crowned Babbler

One species in Australia, the Grey-crowned Babbler, *Pomatostomus temporalis*, has been studied for over 30 years (Councilman and King 1977; King 1980; Brown et al. 1983; Gill and Dow 1985; Robinson 1993; Adam and Robinson 1996; Robinson 2006; Davidson and Robinson 2009; Blackmore and Heinsohn 2011). The geographic range of the Grey-crowned Babbler incorporates approximately two-thirds of the Australian continent, encompassing much of northern and eastern Australia (Fig. 1.1). A biogeographical barrier in north-eastern Australia divides the distributions of the (only) two subspecies *P. temporalis temporalis* and *P. temporalis rubeculus* (Fig. 1.1). The Grey-crowned Babbler lives in social groups consisting of a dominant breeding pair and up to a dozen or more philopatric offspring of both sexes (Brown and Brown 1981).

Initial research focused on its cooperative-breeding system and it was one of the first cooperatively-breeding species to receive intensive research attention globally (Brown et al. 1978; Councilman 1979; King 1980; Brown et al. 1983). Later studies of the Grey-crowned Babbler included an assessment of its social structure, habitat requirements and population genetic structure (Johnson and Brown 1980; Edwards 1993a; Blackmore
Most research on this species has studied populations in the north and north-eastern areas of their distribution (Queensland and northern New South Wales (NSW)), and has assessed populations in contiguous habitat (Edwards 1993a; Blackmore and Heinsohn 2007; Eguchi et al. 2007; Blackmore and Heinsohn 2008).

Approximation of the biogeographical break between subspecies is indicated with a thick black line on the Australian mainland. *P.t.temporalis* range in New Guinea is indicated by an undulating line in the southern part of the island.

Figure 1.1 Global range of the Grey-crowned Babbler depicting the range extent for *Pomatostomus temporalis rubeculus* and *P.t.temporalis*.
Although the species is generally monogamous, it avoids inbreeding within a closely related neighbourhood by pairing with non-related individuals from external groups (Blackmore and Heinsohn 2008). No evidence of genetic isolation-by-distance greater than a local neighbourhood or group territory (<1.5 km) was apparent in a population occurring in northern NSW and, uncommon amongst avian species, the Grey-crowned Babbler shows a lack of sex-biased dispersal (Blackmore et al. 2011).

Studies of the species in the southernmost areas of its range have provided some insight into habitat use (Robinson 1993; Adam and Robinson 1996; Robinson 2006; Radford 2008), but no investigations of habitat fragmentation effects on habitat quality, movement ecology, population genetic structure or gene flow have been undertaken. This thesis focuses on the eastern subspecies, *P. t. temporalis*, and specifically the southernmost population now located in Victoria. This species was once common throughout much of eastern Australia (Department of Environment and Heritage 2013), but has experienced major population declines and extinctions in parts of its southern range (Robinson 1993; Department of Environment and Heritage 2013). Major drivers of the demise of populations are considered to be the loss, fragmentation and degradation of its habitat (Environment Australia 2000; Blackmore 2006). In Victoria, Grey-crowned Babbler groups persist mostly in small areas of remnant woodland habitat, which occur as fragmented patches and linear strips of roadside or riparian vegetation surrounded by agricultural production (Robinson 2006). These remnants are vestiges of former widespread and connected woodland ecosystems.
1.6 The theme and question focus of this thesis

The objective of this thesis is to investigate the effects of habitat loss and fragmentation on ecological and biological parameters of the Grey-crowned Babbler, which has experienced recent major range contraction and population declines. Using the parameters of habitat use, genetic diversity, population genetic structure and dispersal patterns, investigations are undertaken on 72 family groups across three broad regions in the southern periphery of the species range in south-eastern Australia. I utilise landscape ecology and landscape genetics, along with three decades of census and survey data collected on Grey-crowned Babbler groups across this same area (Robinson 1993; Tzaros 1995; Tzaros 2001; Lacey unpub. data) to specifically elucidate:

1. if demographic parameters (group size and breeding success) can provide biologically relevant measures of habitat quality for the Grey-crowned Babbler amongst different regions, population sizes, isolation levels and habitat types;

2. if population substructure exists at the contemporary southern range edge for the species which might be explained by local-scale isolation-by-distance effects, and if evidence of sex-biased dispersal is present;

3. if landscape-scale habitat loss and fragmentation (i.e. landscape dis-connectivity) influences spatial and temporal patterns of genetic diversity and demographic history within populations, such as gene flow and signatures of recent bottlenecks;

4. if either a landscape-level isolation-by-distance or isolation-by-resistance
model is more supported to explain the genetic distances of individuals
across the study area, and if correlated model/s are further pronounced
in the more philopatric sex, and;

5. areas of landscape-scale structural connectivity that aligns with
functional connectivity patterns (e.g. contemporary population genetic
substructure, gene flow rates, dispersal direction) and highlight
important dispersal pathways for conservation management of this
species.

This thesis includes four data chapters (Chapters 2, 3, 4 and 5), which have been
written as stand-alone bodies of publishable work. This approach means that there is
some repetition in the Introduction and Methods sections in each chapter.

The first data chapter, Chapter 2 (Stevens et al. 2015), introduces the Grey-
crowned Babbler and provides background for the current issues that the species faces
in its southern edge-of-range distribution. In this chapter, I examine factors that
potentially influence habitat quality for the Grey-crowned Babbler. I use data from 72
sites known to be occupied by Grey-crowned Babbler groups and a stratified sampling
design incorporating different spatial scales and isolation levels, to investigate whether
demographic parameters (group size and breeding success) can provide biologically
relevant measures of habitat quality for the species. I identify a priori factors believed to
influence territory quality, of which group size and breeding success were modeled
against to identify predictors of habitat quality. This chapter has been published
(Stevens et al. 2015) in PLoS One.
Building on an ecological understanding of Grey-crowned Babbler habitat use at site- and landscape-levels, I introduce a landscape genetics approach for the remaining data chapters 3, 4 and 5. By incorporating a genetics framework, this research is able to draw comparisons with recent genetic studies conducted on the Grey-crowned Babbler in different ecosystems (Eguchi et al. 2007; Blackmore and Heinsohn 2008).

In Chapter 3, the first of the genetic studies, I investigate population genetic structure and connectivity across the highly fragmented southern range of the Grey-crowned Babbler. Specific aims are to investigate: 1) if population structure existed at the contemporary southern range-edge of the species; 2) if population structure can be explained by isolation-by-distance effects; and 3) if evidence of sex-biased dispersal existed. I compare findings to those of similar studies conducted on the Grey-crowned Babbler in areas of continuous habitat cover to determine if more extensive habitat loss and fragmentation in southern parts of the species’ range may be reducing genetic connectivity. This chapter is in press for publication in Emu.

Chapter 4 assesses the data for a genetic bottleneck signature within study populations, and to detect gene flow discordance which may imply habitat fragmentation effects. Bayesian and coalescent-based analyses are used to estimate contemporary and historic migration levels and direction, as well as individuals’ ancestry over recent generations (i.e. up to three generations). I also estimate effective population size and models of demographic history.

In the final data chapter (Chapter 5), I investigate if the Grey-crowned Babbler is supported from structural connectivity across the study landscape to disperse across
large landscape-level distances and what habitat features are assisting in the genetic
signature across their southern range. I do this by building a suite of landscape
connectivity models using electric current theory as the construct. Plausible models
include isolation-by-distance and isolation-by-resistance models for comparison and
contrast between the models. Additionally, by modelling landscape connectivity I
identify important landscape-level mechanisms facilitating dispersal of the species in
their southern range periphery to inform conservation management.

These data chapters combine to enable insight into the role of anthropogenic
landscape modification in influencing the population and genetic structure of this
sedentary species. The final chapter (Chapter 6) presents a synthesis of these results
and discusses the implications for conservation and management of the Grey-crowned
Babbler. In conclusion, recommendations for species and habitat management, and
suggestions for future research to complement the work presented here, are given.

These recommendations aim to assist this species, and other declining woodland
species, affected by habitat loss and fragmentation in an age of climatic uncertainty.
What determines habitat quality for a declining woodland bird in a fragmented environment: the Grey-crowned Babbler *Pomatostomus temporalis* in south-eastern Australia?

A Grey-crowned Babbler family group utilising roadside habitat, often the only remnant habitat readily available in many parts of their southern distribution.

This chapter has been published as:

2.1 Abstract

Understanding what constitutes high quality habitat is crucial for the conservation of species, especially those threatened with extinction. Habitat quality frequently is inferred by comparing the attributes of sites where a species is present with those where it is absent. However, species presence may not always indicate high quality habitat. Demographic parameters are likely to provide a more biologically relevant measure of quality, including a species’ ability to successfully reproduce. We examined factors believed to influence territory quality for the Grey-crowned Babbler (*Pomatostomus temporalis*), a cooperatively-breeding woodland bird that has experienced major range contraction and population decline in south-eastern Australia. Across three broad regions, we identified active territories and determined the presence of fledglings and the size of family groups, as surrogates of territory quality. These measures were modelled in relation to habitat attributes within territories, the extent of surrounding wooded vegetation, isolation from neighbouring groups, and the size of the neighbourhood population. Fledgling presence was strongly positively associated with group size, indicating that helpers enhance breeding success. Surprisingly, no other territory or landscape-scale variables predicted territory quality, as inferred from either breeding success or group size. Relationships between group size and environmental variables may be obscured by longer-term dynamics in group size. Variation in biotic interactions, notably competition from the Noisy Miner (*Manorina melanocephala*), also may contribute. Conservation actions that enhance the number and size of family
groups will contribute towards reversing declines of this species. Despite associated challenges, demographic studies have potential to identify mechanistic processes that underpin population performance; critical knowledge for effective conservation management.

2.2 Introduction

Effective conservation requires an understanding of what constitutes suitable habitat for species of concern (Carroll et al. 2001; Manning et al. 2004; Whittingham et al. 2005). Habitat provides resources needed for survival and reproduction, including food, shelter and nesting sites. The availability of such resources typically varies spatially in response to broad environmental gradients (Loyn et al. 2001; Wintle et al. 2005).

Studies that investigate the habitat requirements of species often compare ‘habitat’ (species present) with ‘non-habitat’ (species absent). Such presence/absence studies form the basis of most species distribution models (Guisan and Zimmermann 2000; Elith et al. 2006). However, the presence of a species at a location does not necessarily indicate habitat of high quality (Schlaepfer et al. 2002; Kuussaari et al. 2009; Holland and Bennett 2010). For example, population ‘sinks’ represent areas of habitat where individuals of a species occur but reproductive output falls below the threshold required for a self-sustaining population (Pulliam 1988).

Anthropogenic land-use and landscape change also profoundly affect the availability and quality of habitat for many species (Lindenmayer and Fischer 2006; Holland and
Bennett 2007; Uezu and Metzger 2011) and act to amplify the variation in habitat quality associated with environmental gradients. Consequently, conservation management is likely to be more effective when it is possible to move beyond presence/absence comparisons and focus on more biologically relevant indicators of habitat quality. Demographic parameters, such as population size and reproductive output, represent two such indicators. Comparing demographic parameters across spatially separated populations will likely provide valuable insights into relative population performance, which in turn can be used to infer habitat quality (Holland and Bennett 2010). Reducing the ambiguity of what constitutes high quality habitat for species will become increasingly important as more areas are subjected to human-induced disturbance (Lindenmayer and Fischer 2006).

Comparing population demographic parameters across broad spatial scales is considerably more challenging and time consuming than conducting presence/absence studies, explaining why studies of population demography are comparatively few. In south-eastern Australia, the habitat requirements of the Grey-crowned Babbler (Pomatostomus temporalis), a threatened species of woodland bird, have been assessed using presence/absence studies (Robinson 1993; Adam and Robinson 1996; Radford 2008). In an effort to shift beyond a framework of habitat and non-habitat to more refined measures of habitat quality, we focus on demographic parameters of this species at occupied sites only. We use the presence of fledglings and family group size as surrogates for territory quality (Brown et al. 1982; Blackmore and Heinsohn 2007). We examine several hypotheses concerning factors that influence the quality of
territories occupied by family groups of the Grey-crowned Babbler. Specifically we test whether reproductive capacity and group size are influenced by: 1) habitat structure and complexity within the territory (used as surrogates for local resource availability e.g. food, shelter, nest sites); 2) the extent of wooded vegetation surrounding the territory (a measure of total habitat available to each group); 3) the degree of isolation from nearby territories (used as a surrogate for habitat fragmentation and exchange of individuals between groups); and 4) the size of the neighbourhood population (used as a surrogate for habitat quality/availability at a broader landscape-level).

2.3 Methods

2.3.1 Study species

The Grey-crowned Babbler is a cooperatively-breeding woodland bird which lives in social groups consisting of a dominant breeding pair assisted by ‘helpers’ (usually previous offspring) (Brown et al. 1983; Blackmore and Heinsohn 2007). Reproduction occurs over an extended season from June to March/April. Grey-crowned Babblers feed on invertebrates taken at ground-level and also from the trunks and foliage of trees and shrubs. They construct numerous large (~40-50 cm) communal roost nests in their territory. At least one brood nest is constructed per breeding season for use by a breeding female (Dow and King 1984). The species is widespread in northern and eastern Australia, but has declined markedly in the southern part of its range where there has been extensive loss, fragmentation, and degradation of native vegetation.
(Robinson 2006). In the south, the Grey-crowned Babbler persists in remnant woodland patches within agricultural landscapes, most often characterised as roadside vegetation separated by unsuitable areas of cleared farmland (Robinson 2006). These remnants are some of the last vestiges of once widespread and connected woodland ecosystems of southern Australia (Lunt and Bennett 2000; Lindenmayer et al. 2010). As a result, the Grey-crowned Babbler is now listed as threatened in two states (Department of Environment and Primary Industries 2012; Department of Environment and Heritage 2013).

2.3.2 Study area

The study area encompasses ~22,250 km² in northern Victoria, Australia, stratified across three regions: west (centred on Kerang, 36.12°S, 143.72°E), south-east (centred on Benalla, 36.55°S, 145.98°E) and north-east (centred on Rutherglen, 36.06°S, 146.46°E) (Fig. 2.1). These three regions support the largest populations of the Grey-crowned Babbler in Victoria. A gradient in mean annual rainfall occurs across this region (Kerang: 387 mm; Rutherglen: 588 mm; Benalla: 651 mm) (Bureau of Meteorology 2012). Above average rainfall occurred in 2010 and 2011, and atypical flooding events occurred in early 2011, with large areas in the west affected by floodwaters for several months.
Native vegetation in the study area consists primarily of eucalypt woodlands that vary in composition according to soil type and rainfall. Since the mid-1800’s, some 85% of native vegetation within this area has been cleared for agriculture (Environment Conservation Council 2001), with little vegetation remaining in its natural state. In the west, woodlands are dominated by Black Box (*Eucalyptus largiflorens*) with a sparse understorey of flood-tolerant vegetation such as Lignum (*Muehlenbeckia florulenta*) in wetter sites, and *Acacia* spp. in drier areas. In both the south-east and north-east regions, woodland remnants are dominated by single-species stands of Grey Box (*E.*
microcarpa), with smaller areas of other single or mixed-eucalypt species. Mid-storey vegetation consists of shrubs such as Gold-dust Wattle (Acacia acinacea) and Golden Wattle (A. pycnantha). All regions contain introduced grasses and some introduced mid-storey species such as Boxthorn (Lycium ferocissimum) and Peruvian Peppercorn (Schinus molle).

2.3.3 Site selection

Study sites (i.e. occupied territories) were selected based on the known occurrence of Grey-crowned Babblers (Tzaros 1995; 2001). Sites were stratified by region (west; south-east; north-east), with 24 territories in each (n = 72 territories in total, representing 3.6% of known Grey-crowned Babbler territories in Victoria) (Table A1.1). In each region, territories were selected within both large (>30 groups) and small (≤30 groups) neighbourhood populations, with neighbourhoods separated by ≥5 km from other identified neighbourhoods or known groups. Territories were further stratified according to distance to the nearest adjacent territory (near <1 km, n = 47; far ≥1 km, n = 25). Call playback was employed to confirm the presence of Grey-crowned Babblers at all sites. An on-ground search using call playback was conducted in areas of适合 habitat within a 2 km radius of each study territory to determine distances to adjacent groups.

Territory size and configuration differed. Most territories (53%) were located in linear strips of roadside vegetation, 40-60 m in width. Thirty-three per cent were in small patches <5 ha, consisting of remnant vegetation, or revegetated linear strips within farmland adjacent to roadside vegetation. The remaining 14% of territories were
in native woodland patches >5 ha, two of these incorporating private gardens which abutted large woodland patches.

2.3.4 **Bird surveys**

Surveys of Grey-crowned Babblers spanned an entire breeding season, June 2010 to April 2011 (except for two territories that were surveyed in the following 2011/12 breeding season). The breeding female of each territory displays reproductive behaviour several weeks before egg laying. A nesting cycle typically results in 2-4 eggs being laid and surviving hatchlings generally fledge after ~8 weeks (Blackmore and Heinsohn 2007). Breeding success was therefore measured by visiting every territory at approximately eight week intervals over the entire breeding season, to determine whether recently fledged young were present (Table A1.1). In this way, we accounted for multi-brooding events within a single season. Although fledglings may not have been recorded on some visits at some sites due to predation, we considered predation of all fledged offspring at a site over an entire breeding season unlikely. Recently fledged young were identified from adults by their small size, obvious grey ear coverts, dark brown eyes and begging behaviour; characteristics retained up to 0.5 years of age (Counsilman and King 1977). Mean group size (Table A1.1) was determined from surveys recorded during each visit.
2.3.5 Vegetation surveys

Vegetation and habitat features were assessed at each site in each of three quadrats (50 X 20 m): one was located at an active roost nest, while the remaining two were located at points where Grey-crowned Babblers were observed foraging. This ensured that vegetation assessments were centred on active areas of each territory. Data collected in each quadrat included the number of shrubs >1 m height, percent cover of lignum, number of logs >30 cm diameter, number of stumps, and the number and species of live trees classified into size classes (<10, 10-30, 30-60, 60-90 and >90 cm diameter at breast height (DBH)). The quadrat midline provided a 50 m transect along which additional data were collected. A measuring pole was placed perpendicular to the ground at 5 m intervals along the transect (n = 11 points). Ground substrates that the pole touched were recorded (i.e. short grass <10 cm, herbs, leaf litter and bare ground), as was vegetation touching the pole in each 10 cm height class from 10-100 cm (i.e. long grass >10 cm and shrubs). The habitat attributes of each territory were then derived by calculating the mean value from the three quadrats.

2.3.6 Regional habitat comparisons

We used nonmetric multi-dimensional scaling (nMDS) ordination to compare the vegetation structure at sites between regions, using a Bray-Curtis measure of similarity, implemented in PRIMER (Clarke and Gorley 2006). We tested whether variation in vegetation structure between regions was greater than variation within regions using analysis of similarity (ANOSIM), and compared structural variation within sites for each region using SIMPER (Clarke and Warwick 2001).
2.3.7 Variable selection for modelling

We used two dependent variables to investigate the response of the Grey-crowned Babbler to site and landscape characteristics: 1) breeding success of groups, as indicated by fledgling occurrence; and 2) group size (averaged across three surveys). A set of explanatory variables was derived to represent variation in habitat features across study territories. Direct measurement of resource availability (e.g. ground and bark invertebrates which represent the bulk of the Grey-crowned Babbler diet) is not logistically feasible across an extensive study area such as that used here. Instead, data were obtained for local habitat characteristics considered to be reliable surrogates of resource availability (Taylor 2008). From the habitat data available, litter cover and the number of shrubs, large trees (>60 cm DBH), stumps and large logs (≥30 cm diameter) were selected for inclusion in models (Table 2.1). These variables were considered reliable surrogates for key resources including food, foraging substrates, and shelter/nesting sites (Brown et al. 1983; Robinson 1993; Radford 2008). A further four explanatory variables were included due to their likely influence on the dependent variables at the landscape-scale (Table 2.1). Local tree cover was the area (ha) of wooded cover (native wooded vegetation) within a 300 m radius of each territory, and represents the amount of habitat available to each study group. Territory isolation was represented by two variables: the average distance to neighbouring territories, and the area (ha) of wooded cover within a 1 km radius of territories. These variables represent the degree of local habitat fragmentation and act as surrogates for exchange of individuals between groups. Finally, local neighbourhood size (large: >30 groups; small:
≤30 groups) was included as a categorical variable to represent habitat quality/availability at a broader landscape-level. The last four variables are important to consider since they represent habitat availability and fragmentation; factors considered likely to have a strong influence on species with complex social systems such as cooperatively-breeding birds (Blackmore et al. 2011).

2.3.8 Model development and selection

Explanatory variables were grouped to represent distinct hypotheses regarding influences on breeding success and group size: 1) habitat structure variables (surrogates for food supply, shelter and nest sites); 2) local tree cover (local habitat availability); 3) territory isolation (local habitat fragmentation and exchange of individuals); and 4) local neighbourhood size (landscape-level habitat availability/quality). Group size was included as an additional hypothesis for the ‘fledgling occurrence’ response variable as it was likely to be an important predictor of breeding success. A single model was developed for each hypothesis group, and all possible combinations of groups were also assessed (total models: breeding success, \( n = 31 \); group size, \( n = 15 \)). The nature of the relationship between response and explanatory variables was explored using scatterplots, component-and-residual plots, and the fit of simple univariate models. Explanatory variables (Table 2.1) were standardised for all generalised linear mixed-models and transformed to linearise relationships as required. Continuous explanatory variables were not highly correlated \( (r \leq 0.4) \).
Table 2.1 Explanatory variables for models of breeding success and group size for the Grey-crowned Babbler.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Type</th>
<th>Range</th>
<th>Mean</th>
<th>Group size analysis</th>
<th>Breeding success analysis</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf litter</td>
<td>Continuous</td>
<td>0.23-0.96</td>
<td>0.67</td>
<td>Yes</td>
<td>Yes</td>
<td>Average percent cover of leaf litter for three quadrats (surrogate for availability of food and foraging substrates)</td>
</tr>
<tr>
<td>Trees</td>
<td>Continuous</td>
<td>0-10</td>
<td>2.70</td>
<td>Yes</td>
<td>Yes</td>
<td>Total number of trees with trunks &gt;60 cm DBH from three quadrats (surrogate for availability of food and foraging substrates)</td>
</tr>
<tr>
<td>Shrubs</td>
<td>Continuous</td>
<td>0-344</td>
<td>49.25</td>
<td>Yes</td>
<td>Yes</td>
<td>Total number of shrubs &gt;1 m height and trees &lt;10 cm DBH from three quadrats (surrogate for availability of food, foraging substrates, shelter/nesting sites)</td>
</tr>
<tr>
<td>Stumps</td>
<td>Continuous</td>
<td>0-36</td>
<td>6.39</td>
<td>Yes</td>
<td>Yes</td>
<td>Total number of stumps from three quadrats (surrogate for availability of food and foraging substrates)</td>
</tr>
<tr>
<td>Logs</td>
<td>Continuous</td>
<td>0-15</td>
<td>2.87</td>
<td>Yes</td>
<td>Yes</td>
<td>Total number of logs ≥30 cm diameter from three quadrats (surrogate for availability of food and foraging substrates)</td>
</tr>
<tr>
<td>Group size&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Continuous</td>
<td>2-12</td>
<td>5.59</td>
<td>No</td>
<td>Yes</td>
<td>Average number of individuals in a territory</td>
</tr>
<tr>
<td>Neighbourhood size</td>
<td>Categorical</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Large (&gt;30 territories in population); small (≤30 territories) with ≥5 between populations (indicator of habitat quality/availability at landscape level)</td>
</tr>
<tr>
<td>Group isolation&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Continuous</td>
<td>0.00-4396.70</td>
<td>1008.80</td>
<td>Yes</td>
<td>Yes</td>
<td>Distance (m) from the nearest neighbour territory (indicator of local fragmentation and exchange of individuals between groups)</td>
</tr>
<tr>
<td>Local tree cover&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Continuous</td>
<td>0.41-19.04</td>
<td>7.25</td>
<td>Yes</td>
<td>Yes</td>
<td>Tree cover (ha) within a 300 m radius of where territories occurred of habitat available to each study group)</td>
</tr>
<tr>
<td>Landscape tree cover</td>
<td>Continuous</td>
<td>3.04-185.45</td>
<td>50.85</td>
<td>Yes</td>
<td>Yes</td>
<td>Tree cover (ha) within a 1 km radius of where territories occurred of local habitat fragmentation and exchange of individuals between groups</td>
</tr>
<tr>
<td>Region&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Categorical</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Three regions: west; south-east; north-east</td>
</tr>
</tbody>
</table>

<sup>a</sup> Log<sub>10</sub> transformed for breeding success models only; <sup>b</sup> Range and mean values provided for untransformed data; <sup>c</sup> Included as an explanatory variable in the generalised linear model for group size; included as a random term in all other models
Hypotheses regarding breeding success (presence/absence of fledglings) were tested using generalised linear mixed-models employing a binomial error distribution. Study region (west, south-east, north-east) was included as a random term to account for possible non-independent error structures due to clustering of Grey-crowned Babbler territories (Zuur et al. 2009). This approach facilitates the identification of explanatory variables that influence the dependent variable irrespective of study region. Only sites where the age of all group members was identified throughout the 2010/11 breeding season were included in analyses of breeding success (n = 63).

Analyses of group size occurred in two stages. First, we were interested to know whether group size differed by region. A generalised linear model was constructed with region as the sole explanatory variable. Second, our four key hypotheses were tested using generalised linear mixed-models with region as a random term. A Poisson error distribution was employed in both cases. No over-dispersion was detected in Poisson models.

Akaike’s information criterion corrected for small sample size (AICc) was computed for all models representing the key hypotheses, as were AICc weights (wi; evidence in favour of a given model being the best of those considered) (Burnham and Anderson 2002). Akaike weights were summed (to a value of 0.95) to generate a 95% confidence set for the best model (Burnham and Anderson 2002). Model-averaging was conducted where no standout model (wi >0.9) was identified (Burnham and Anderson 2002). Explanatory variables were considered to be an important influence on the dependent
variable where the 95% confidence interval (standard error multiplied by 1.96) for model-averaged coefficients did not overlap zero. Model fit was assessed in various ways owing to the different modelling approaches used: 1) conditional and marginal pseudo-$R^2$ (binomial glmm; (Nakagawa and Schielzeth 2013)); 2) adjusted $D^2$ (Poisson glm; (Guisan and Zimmermann 2000)); and 3) likelihood-ratio based pseudo-$R^2$ (Poisson glmm; (Barton 2012)). Residual plots were inspected to ensure that model structures were appropriate for the data. All statistical analyses were performed in R v 3.0.2 (R Core Team 2013) using the packages car v 2.0-19 (Fox and Weisberg 2011), lme4 v 1.0-5 (Bates et al. 2013), AICcmodavg v 1.35 (Mazerolle 2012), and MuMIn v 1.9.13 (Barton 2012).

2.4 Results

2.4.1 Comparison of habitat attributes within and between regions

Habitat attributes of territories differed between regions (ANOSIM Global $R = 0.279$; $p < 0.001$) (Table A1.2) with the largest differences being between the west and the north-east ($R = 0.417$, $p < 0.001$), and the west and south-east ($R = 0.364$, $p < 0.001$). Habitat attributes did not differ between the south-east and north-east ($R = 0.030$, $p = 0.112$) (Fig. A1.1). The cover of short grass and leaf litter was a major contributor to regional differences, with reduced cover of these elements in territories in the west. The same variables contributed most to between-site similarities within regions (Table A1.3).
2.4.2 Breeding success (fledgling presence/absence)

Two-thirds of monitored Grey-crowned Babblers produced fledglings during the 2010/11 breeding season. Of the models considered to account for fledgling occurrence, two had substantial support ($\Delta_i < 2$) (Table 2.2). Grey-crowned babblers group size was included in all five models in the 95% confidence set and was the single explanatory variable in the first ranked model, accounting for 54% of variance in the data (both marginal and conditional $R^2$ values = 54%). Model averaging revealed group size to be the only explanatory variable to have an important influence on the presence of fledglings (coefficient = 3.93; CI = 2.03, 5.83) (Fig. 2.2a). The probability of occurrence of fledglings is predicted to increase rapidly as group size increases from two to seven (Fig. 2.3). Once a group size of seven or more is attained, the probability of fledglings being detected exceeds 90%.
Table 2.2 Model-selection results for breeding success (fledgling presence/absence) and mean group size of the Grey-crowned Babbler.

The models shown are those within the 95% confidence set. Included are the number of parameters (df), log-likelihood values ($\text{Log}(l)$), AIC$_c$ values, Akaike differences ($\Delta_i$), and Akaike weights ($w_i$).

<table>
<thead>
<tr>
<th>Model name</th>
<th>Model components</th>
<th>df</th>
<th>Log(l)</th>
<th>AIC$_c$</th>
<th>$\Delta_i$</th>
<th>$w_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding success</td>
<td>Group size</td>
<td>3</td>
<td>-26.2</td>
<td>58.8</td>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Group size and neighbourhood size</td>
<td>4</td>
<td>-25.8</td>
<td>60.2</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Group size and local tree cover</td>
<td>4</td>
<td>-26.0</td>
<td>61.0</td>
<td>2.3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Group size, neighbourhood size and local tree cover</td>
<td>5</td>
<td>-25.8</td>
<td>62.6</td>
<td>3.8</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Group size and isolation</td>
<td>5</td>
<td>-25.8</td>
<td>62.7</td>
<td>3.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Group size</td>
<td>Local tree cover</td>
<td>3</td>
<td>-156.3</td>
<td>319.0</td>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Neighbourhood size</td>
<td>3</td>
<td>-156.8</td>
<td>320.0</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Local tree cover and neighbourhood size</td>
<td>4</td>
<td>-156.3</td>
<td>321.2</td>
<td>2.2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Group isolation</td>
<td>3</td>
<td>-156.4</td>
<td>321.5</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Local tree cover and group isolation</td>
<td>4</td>
<td>-156.2</td>
<td>323.4</td>
<td>4.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Figure 2.2 Model-averaged coefficients and associated 95% confidence intervals for explanatory variables included in models of (a) breeding success (fledgling presence/absence); and (b) group size, of the Grey-crowned Babbler.
Grey shading represents the 95% confidence interval for predicted values. Predictions were generated from model-averaged parameter estimates of generalized linear mixed models.

Figure 2.3 Predicted probability of occurrence of Grey-crowned Babbler fledglings as a function of average group size.
2.4.3 Group size

Of the 72 babbler groups monitored, group size ranged from 2-12 individuals (mean = 5.6) (Table A1.1). Eight groups (11%) varied in group size during the study: four increased by 1-2 individuals while four decreased by 1-2 individuals.

Study region was an important influence on group size. Compared with the south-east region, group size was larger in the west (coefficient = 0.29; 95% CI = 0.05, 0.53). There was no difference in group size between the south-east and north-east regions (coefficient = 0.09; 95% CI = -0.17, 0.35). Average group size in the west was 6.5 (± 0.5 se), while in the south-east it was 5.3 (± 0.4 se) and in the north-east 4.9 (± 0.5 se). The $D^2$ value for this model was 7.0%, indicating that much variance remained unexplained.

Of the models considered that relate our four key hypotheses to group size, two had substantial support ($\Delta_i < 2$), while the 95% confidence set comprised five models (Table 2.2). The first ranked model included only local tree cover and had a pseudo-$R^2$ value of 2.0%. Local tree cover occurred in three of the five models in the 95% confidence set (Table 2.2). However, model averaging indicated that none of the explanatory variables considered were an important influence on group size (i.e. 95% confidence intervals for all model-averaged coefficients overlapped zero) (Fig. 2.2b).
2.5 Discussion

While presence/absence studies provide a coarse measure of habitat v’s. non-habitat areas, studies of demographic parameters have the potential to reveal fine-scale variation in habitat quality across occupied sites. Here, we built on knowledge of core habitat preferences of the Grey-crowned Babbler derived from presence/absence studies to investigate whether there are particular attributes, at both site- and landscape-scales, associated with the quality of the species’ territories.

Overall, 58% of Grey-crowned Babbler groups were observed with one or more fledglings during the study. The probability of fledgling occurrence was positively correlated with group size. The number of available carers is known to positively influence reproductive success in a range of cooperatively-breeding taxa (e.g. birds: Mumme 1992; fish: Balshine et al. 2001; mammals: Clutton-Brock et al. 2001) through enhancing food provisioning, parental and/or sentry duties, and predator defence (Clutton-Brock et al. 1998; Hatchwell 1999; Clutton-Brock 2002). The number of non-breeding helpers also has been shown to be important for the Grey-crowned Babbler, with larger groups having higher fledging success (Brown et al. 1982; Blackmore and Heinsohn 2007).

As a response variable, the size of Grey-crowned Babbler groups was influenced by study region, with group size being larger in the west compared to both the south-east and north-east regions. However, the vegetation attributes that differed most between territories in different regions (e.g. litter cover) were
not useful predictors of group size and, controlling for regional effects, no site- or landscape-scale variables were found to influence group size. Agricultural land-use in the west is largely given to cropping, while in the two eastern regions there is both cropping and grazing. Fine-scale variation in landscape structure associated with variation in land-use, such as differential retention of paddock trees (Gibbons and Boak 2002; Maron 2005), may contribute to regional differences in group size.

The lack of stronger relationships between demographic parameters and site attributes is surprising, given that we chose attributes from a suite of variables previously hypothesized as being important indicators of territory quality for the Grey-crowned Babbler (Robinson 1993; Radford 2008; Davidson and Robinson 2009). One explanation is that by surveying occupied sites only, spatial variation in key resources across studied territories may not have been sufficient to elicit consistent demographic change. Almost all territories were located in relatively open woodland vegetation and provided some large trees (>60 cm DBH), patchy tall shrubs or eucalypt regeneration, larger logs (>30 cm diameter), leaf litter and a sparse ground layer (Table 2.1). Grey-crowned babblers may not occupy sites where some or all of these characteristics are at low levels or absent (Adam and Robinson 1996; Radford 2008). Further, this species has declined markedly across this region (Robinson 2006) and it is possible that the species is now restricted largely to remaining areas of high quality habitat.

The number of Grey-crowned Babblers within a group is dynamic (Brown et al. 1983; Blackmore and Heinsohn 2007; Blackmore et al. 2011) and temporal
changes in group size may obscure relationships with habitat attributes. Groups of more than 8-10 individuals are uncommon with territory fission taking place as numbers increase, resulting in more groups of fewer individuals (Blackmore et al. 2011). The size of a group also may decrease when offspring disperse or when a breeder dies and group disintegration occurs (Blackmore et al. 2011). Conversely, group size may increase with successful reproduction (Brown et al. 1983) and with the immigration of individuals from nearby groups (Blackmore et al. 2011). These dynamic processes may occur irrespective of the attributes of a site. Thus, it is plausible that groups of markedly different size may occupy territories of the same quality; in high quality territories a small group may be the result of recent fission, whilst a large group may be the result of recent reproductive success. Concurrent genetic research has identified an isolation-by-distance pattern amongst individuals in the study region, indicating that dispersal is locally restricted in fragmented habitat by between-group distances, and/or fragmentation effects. Thus, variation in group size may also be a consequence of restricted offspring dispersal.

Another possible reason for the lack of clear predictors of territory quality is that biotic interactions, such as competition and predation, may vary in strength across the study area and obscure relationships between breeding success, group size and environmental attributes. An aggressive interspecific competitor, the Noisy Miner (Maron et al. 2013), was present at every Grey-crowned Babbler territory, and was observed to frequently harass and attack Grey-crowned Babblers as they foraged. We did not quantify the abundance of Noisy Miners at
each territory, and so could not examine their influence on group size or reproductive success. However, the aggressive actions of this avian competitor, especially where they occur at higher densities, may influence the demography of the Grey-crowned Babbler. Avian predators of nests or fledglings, such as butcherbirds (*Cracticus* spp.), currawongs (*Strepera* spp.), ravens (*Corvus* spp.) and the Laughing Kookaburra (*Dacelo novaeguineae*), are common in small remnants and linear strips (Ford *et al*. 2001; Robertson *et al*. 2014), and were regularly encountered at Grey-crowned Babbler territories during this study. Geographic variation in the abundance or impact of predators (e.g. more frequent occurrence of the Pied Currawong (*S. graculina*) in the east of our study area (Department of Conservation, Forests and Lands and the Royal Australasian Ornithologists Union 1987)), may also mask relationships between the demography of the Grey-crowned Babbler and indicators of habitat quality. The role of interspecific competition and predation as influences on the population performance of the Grey-crowned Babbler requires further research.

Our results highlight the importance of conservation actions that enhance the size of Grey-crowned Babbler family groups to facilitate higher probabilities of breeding success for this declining species. Several management projects within the study area show promise in achieving this goal (Robinson 2006; Thomas 2009). They involve targeted restoration at a landscape scale with three main components: protecting and maintaining woodland remnants of known habitat for the species (especially numerous large old trees); expanding the overall amount of wooded vegetation through complementary revegetation and
regeneration; and increasing the connectivity of wooded habitats at the landscape scale (Robinson 2006; Thomas 2009). Where such work has been undertaken there is evidence that the number of Grey-crowned Babbler groups has either stabilised or increased (Vesk et al. 2015), and that group size is dynamic over time. Long-term monitoring of family groups and group size dynamics, in tandem with an assessment of the impacts of competition and predation, will be of great value in more clearly pinpointing the environmental attributes that indicate territory quality and enhanced breeding success.

Studies of population demography typically are more labour intensive and time consuming than presence/absence studies. Strong relationships between demographic parameters and explanatory variables can also be more difficult to detect, especially when only occupied sites are surveyed. However, studies of population processes can lead to the development of new hypotheses and research questions, as in this study. Demographic studies have the potential to identify the mechanistic processes that underpin population performance in human modified landscapes, and knowledge of such processes is critical for effective conservation management.
**Table 2.3 Authorship statement for Chapter 2**

1. **Details of publication and executive author**

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>Title of Publication</th>
<th>Publication details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kate Stevens</td>
<td>What determines habitat quality for a declining woodland bird in a fragmented environment: the Grey-crowned Babbler <em>Pomatostomus temporalis</em> in south-eastern Australia?</td>
<td>PLoS ONE</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/Institute/Division if based at Deakin; Organisation and address if non-Deakin</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kate Stevens</td>
<td>School of Life and Environmental Sciences</td>
<td><a href="mailto:kpst@deakin.edu.au">kpst@deakin.edu.au</a></td>
</tr>
</tbody>
</table>

2. **Inclusion of publication in a thesis**

<table>
<thead>
<tr>
<th>Is it intended to include this publication in a higher degree by research (HDR) thesis?</th>
<th>Yes</th>
<th>If Yes, please complete Section 3 if No, go straight to Section 4.</th>
</tr>
</thead>
</table>

3. **HDR thesis author’s declaration**

<table>
<thead>
<tr>
<th>Name of HDR thesis author if different from above. (If the same, write “as above”)</th>
<th>School/Institute/Division if based at Deakin</th>
<th>Thesis title</th>
</tr>
</thead>
<tbody>
<tr>
<td>As above</td>
<td>School of Life and Environmental Sciences</td>
<td>Species conservation in fragmented landscapes: Implications for the Grey-crowned Babbler</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

- conception of the project, 85%
- the design of experimental protocol, 70%
- data collection, 99%
- analysis, 70%
- drafting the manuscript, 100%
- revising it critically for important intellectual content, 50%

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

Signature and date: 15 July 2015

4. **Description of all author contributions**

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greg J. Holland</td>
<td>analysis, revising it critically for important intellectual content</td>
</tr>
<tr>
<td>School of Life and Environmental Sciences, Deakin University, Melbourne, Victoria, Australia, and; Department of Ecology, Environment and Evolution, La Trobe University, Melbourne, Victoria, Australia</td>
<td></td>
</tr>
<tr>
<td>Rohan H. Clarke</td>
<td>conception of the project, the design of experimental protocol, data collection,</td>
</tr>
<tr>
<td>School of Biological Sciences, Monash University, Melbourne, Victoria, Australia</td>
<td></td>
</tr>
</tbody>
</table>

40
<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raylene Cooke</td>
<td></td>
<td></td>
</tr>
<tr>
<td>School of Life and Environmental Sciences, Deakin University, Melbourne, Victoria, Australia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andrew F. Bennett</td>
<td></td>
<td></td>
</tr>
<tr>
<td>School of Life and Environmental Sciences, Deakin University, Melbourne, Victoria, Australia, and; Department of Ecology, Environment and Evolution, La Trobe University, Melbourne, Victoria, Australia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Author Declarations
I agree to be named as one of the authors of this work, and confirm:
- that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
- that there are no other authors according to these criteria,
- that the description in Section 4 of my contribution(s) to this publication is accurate,
- that the data on which these findings are based are stored as set out in Section 7 below.
If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further consent to the incorporation of the publication into the candidate’s HDR thesis submitted to Deakin University and, if the higher degree is awarded, the subsequent publication of the thesis by the university (subject to relevant Copyright provisions).

<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greg Holland</td>
<td></td>
<td>27/10/2015</td>
</tr>
<tr>
<td>Rohan Clarke</td>
<td></td>
<td>18/07/2015</td>
</tr>
<tr>
<td>Raylene Cooke</td>
<td></td>
<td>18/07/2015</td>
</tr>
<tr>
<td>Andrew Bennett</td>
<td></td>
<td>14/12/2015</td>
</tr>
</tbody>
</table>

6. N/A
7. Data storage
The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

<table>
<thead>
<tr>
<th>Data format</th>
<th>Storage Location</th>
<th>Date lodged</th>
<th>Name of custodian if other than the executive author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excel spreadsheets</td>
<td>Deakin University: \student-home-m.its.deakin.edu.au</td>
<td>15/07/15</td>
<td></td>
</tr>
</tbody>
</table>

This form must be retained by the executive author, within the school or institute in which they are based.
If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis with the publication.
Total concentrate of deoxyribonucleic acid (DNA) of sampled Grey-crowned Babbler individual number 067. The small amount of liquid just visible at the base of the container was the culmination of two years of field work and 2 weeks of extraction processes in the laboratory. It likely took many 1000’s of years for the Grey-crowned Babbler to evolve it.

This chapter is currently in press:
Stevens K, Harrisson KA, Clarke RH, Cooke R, and Hogan FE (in press). Genetic structure and sex-biased dispersal of a declining cooperative-breeder, the Grey-crowned Babbler (*Pomatostomus temporalis*), at the southern edge of the species’ range. Emu
3.1 Abstract

Habitat loss and fragmentation can disrupt genetic exchange between populations, reflected in changes to the genetic structure of populations. The Grey-crowned Babbler (*Pomatostomus temporalis*), is a cooperatively-breeding woodland bird, once common and widespread in south-eastern Australia. The species has suffered population declines of >90% across its southern distribution as a result of habitat loss and fragmentation. We investigated patterns of genetic diversity and population structure of the Grey-crowned Babbler in fragmented habitats of its southernmost range. We sampled blood from 135 individual Grey-crowned Babblers from 39 groups across three regions. Genotypic data was used to estimate genetic diversity, population substructure, local relatedness and dispersal patterns. Individuals showed high heterozygosity within regions, and varying numbers of private alleles amongst regions suggested differences in connectivity levels between regions. Four genetic clusters revealed population substructure that is consistent with treeless landscapes acting as strong barriers to gene flow. In contrast to previous studies, we identified a male-biased dispersal pattern and significant isolation-by-distance patterns for females at fine spatial scales. We recommend conservation plans for this species incorporate opportunities to increase and enhance corridor areas to facilitate genetic exchange amongst populations.
3.2 Introduction

The geographic structure of a species range is influenced by a number of biotic (e.g. habitat availability) and abiotic (e.g. climate change) factors. These factors continue to change over time and can exert differing pressures on population demographics (e.g. population size and gene flow) (Donald et al. 2001; Crozier and Dwyer 2006; Wittmer et al. 2007). Small, isolated populations are especially vulnerable to extirpation as a result of environmental stochasticity, demographic effects, such as reduced reproductive rates/increased mortality, and genetic effects, such as a loss of genetic diversity through drift and associated reductions in fitness and evolutionary potential (Epps et al. 2005; Méndez et al. 2014; Duncan et al. 2015). Populations can show a gradual decline in size and connectedness toward their range edge (Guo et al. 2005). Understanding population dynamics at the range extremes for a species can therefore be of value for conservation management purposes.

Genetic diversity and gene flow are processes critical for population viability, evolutionary potential, resistance to disease and reducing the negative effects of inbreeding and genetic drift (Frankham 1996). Habitat loss and fragmentation are frequently associated with loss of genetic diversity, disrupted dispersal and reduced gene flow among populations (Palstra and Ruzzante 2008; Jaquiery et al. 2009). Edge-of-range populations therefore can be genetically impoverished and differentiated due to population decline and loss of genetic connectivity with nearby populations and larger core populations (Lesica and
Allendorf 1995; Alda et al. 2013). Understanding the effects of habitat loss and fragmentation on gene flow and genetic diversity will improve estimates of effective population size and strengthen our capacity to manage population connectivity, both of which are integral to biodiversity conservation (Palstra and Ruzzante 2008).

Species vulnerable to habitat fragmentation include restricted dispersers, habitat specialists and species with complex mating systems, for example, cooperative breeders (Frankham 2005). Sedentary species can be more affected by habitat loss and fragmentation than more mobile species (Amos et al. 2014). Cooperatively-breeding species can also be vulnerable to habitat loss and fragmentation as their breeding success is associated with helpers at the nest (Stevens et al. 2015; Chapter 2). Therefore, to increase the success of patch recolonisation following local extinction, groups (i.e. >2 birds) must recolonise a patch. In Australian ecosystems, habitat loss and fragmentation have modified once connected woodlands into small, isolated remnants within a matrix of farmland (Robinson 1993; Robinson and Traill 1996). Landscapes across southeastern areas have lost up to 85% of native vegetation since the mid-1800s and remnant patches now provide habitat refugia for species vulnerable to fragmentation effects (Ford et al. 2001). Species reliant on woodland habitat have subsequently declined to small, isolated populations at risk of local extirpation (Radford and Bennett 2007). Effective conservation management for vulnerable species experiencing habitat loss and fragmentation relies on understanding the implications of critical population processes for the species,
such as dispersal and changing population sizes.

The Grey-crowned Babbler, \textit{(Pomatostomus temporalis)}, is a cooperatively-breeding woodland bird that is sensitive to the effects of habitat loss and fragmentation (Robinson 1993; Blackmore and Heinsohn 2008; Environment Australia 2011). In cooperatively-breeding systems, offspring from one or more generations delay natal dispersal to help raise future generations of young and local neighbourhoods are characterised by closely-related individuals (Koenig et al. 1992). Offspring of the Grey-crowned Babbler remain as helpers at the nest for up to three years (Blackmore and Heinsohn 2007), and the number of helpers increases the likelihood of breeding success (Stevens et al. 2015; Chapter 2). The global range of the Grey-crowned Babbler incorporates the Trans-Fly Region of southern New Guinea and approximately two-thirds of mainland Australia, encompassing much of the northern and eastern States (Fig. 3.1). A sub-specific boundary exists in north-eastern Australia, delineating the distribution of the subspecies \textit{P. t. temporalis} and \textit{P. t. rubeculus} (Edwards 1993a) (Fig. 3.1). This study is focused on the eastern subspecies \textit{P. t. temporalis} and specifically at the southernmost edges of its range in Victoria (Fig. 3.1). The Grey-crowned Babbler was once common throughout much of eastern Australia (Department of Environment and Heritage 2013), but has suffered population declines >90% in the southern portion of its distribution from the loss and fragmentation of Box and Ironbark woodlands \textit{(Eucalyptus spp.)} (Robinson 1993; Robinson 2006; Environment Conservation Council 2001; Environment Australia 2011). In these southern landscapes they are restricted to roadside or riparian vegetation, small
adjacent remnant woodland patches within farmland (<0.5 ha) and habitat edges of the few existing large (>5 ha) conservation reserves (Robinson 2006).

The Grey-crowned Babbler has been the subject of research for over 30 years (Counsilman and King 1977; Gill and Dow 1985; Robinson 1993; Robinson 2006; Davidson and Robinson 2009). Early studies mainly focused on their cooperative breeding system (Brown et al. 1978; Counsilman 1979; King 1980; Brown et al. 1983), while later studies included genetic methods to investigate social structure, breeding ecology and dispersal (Johnson and Brown 1980; Edwards 1993a; Blackmore 2006; Kawano et al. 2007). Most research has been conducted in the north and north-eastern areas of the species’ distribution, and mostly in contiguous habitat (Edwards 1993b; Blackmore and Heinsohn 2007; Eguchi et al. 2007; Blackmore and Heinsohn 2008). Studies conducted within an 85 km² area of continuous habitat in the Grey-crowned Babbler’s north-central distribution revealed spatial genetic clustering of related individuals at a local scale (<1.5 km), but detected no evidence of an isolation-by-distance effect at larger spatial scales up to 10 km (Blackmore et al. 2011). Dispersal distances recorded from recoveries of marked individuals have typically been short (mean = 1 km), although a few exceptions of longer distances have been recorded (max. = 26 km) (Higgins and Peter 2003; Department of the Environment 2015).

Populations experiencing sudden and/or large population declines and reduced connectivity are at higher risk of local extirpation as a result of genetic drift, stochasticity and inbreeding (Nei et al. 1975; Keller and Waller 2002). It is therefore notable that no genetic studies exist for southern parts of the species’
range. In particular, no investigations have explored population structure and connectivity at the southern edge of their range where they have undergone the largest declines (Robinson 1993; Robinson 2006) and experienced the highest levels of habitat loss and fragmentation across the species distribution (Bradshaw 2012).

Here we investigated population genetic structure and connectivity across the highly fragmented southern range of the Grey-crowned Babbler. Our specific aims were to investigate: 1) if population structure existed at the contemporary southern range-edge of the species; 2) if population structure was explained by isolation-by-distance effects; and 3) if evidence of sex-biased dispersal existed. We compared our findings to those of similar studies conducted in areas of continuous habitat cover to determine if more extensive habitat loss and fragmentation in southern parts of the species’ range may be reducing genetic connectivity.

### 3.3 Methods

#### 3.3.1 Study area, site selection and DNA sampling

The study region encompassed an area of ~22, 250 km$^2$ in north-central Victoria, Australia (Fig. 3.1). We investigated if landscape patterns of tree cover might influence the species genetic connectivity by estimating differences of tree cover between the east and west study regions. Tree-cover pattern and extent was assessed using FRAGSTATS v 4 (McGarigal et al. 2012) for the east and west regions of the study area, separately. FRAGSTATS estimated indices of tree cover
for aggregated and dispersed tree cover patterns and which were calculated from 100 m pixel tree cover rasters using ARCGIS v 10.1 (ESRI, 2010).

Using long-term distribution and survey data (Tzaros 1995; Tzaros 2001; Lacey unpub. data; Robinson unpub.data), multiple sampling sites were selected from three geographic regions at the southern-most range of the Grey-crowned Babbler: west \( (n = 15, \text{ Kerang; Boort}) \), south-east \( (n = 12, \text{ Violet Town; Lurg}) \) and north-east \( (n = 13, \text{ Peechelba; Rutherglen; Chiltern}) \) (Fig. 3.1). Original census data show that these regions represent the three major strongholds of Grey-crowned Babbler in Victoria \( \text{(number of groups: west = 68; south-east = 155; north-east = 40)} \), although small numbers of family groups persist in disparate areas of the landscape between regions \( \text{(Tzaros 1995; Tzaros 2001; Lacey unpub. data; Robinson, unpub.data)} \). At each potential site call playback confirmed the presence of a Grey-crowned Babbler family group and occupancy was verified from nesting activity, while repeat visits provided an estimate of group size. An on-ground search using call playback was conducted in areas of suitable habitat within a 2 km radius of each study territory to determine distance to adjacent groups. All site locations were recorded with a Geographic Positioning System (GPS). An average distance of 980 m separated a study site with its closest neighbouring Grey-crowned Babbler group \( \text{(not necessarily a sampled group)} \), which was measured between group centroids \( \text{(usually a nest)} \). Distances between sample sites within regions differed and ranged from 940 m to 75 km. Distances separating sites between regions also varied and ranged from 34 km between a site in the south-east region and one in the north-east, to 258 km...
between a site in the north-east region and one in the west region.

Grey-crowned Babblers live in family groups typically consisting of 2-12 birds (mean = ~5) and occupy territories of 2-53 ha (Higgins and Peter 2003; Blackmore and Heinsohn 2008). Sampling was conducted during 2010-2012 to incorporate two annual breeding seasons from June to April. Birds were lured with call playback and trapped using mist-nets. Each individual was banded with a metal leg band provided by the Australian Bird and Bat Banding Scheme (ABBBS) and a unique combination of three coloured plastic leg bands for identification in the field. Individuals were weighed and measured and a blood sample (~70 μl) collected from the brachial vein using a VITREX® capillary tube. Blood was transferred to a Whatman FTA Card® and stored at room temperature in paper envelopes. We sampled 135 Grey-crowned Babbler individuals from 39 sites (family groups). The number of birds sampled from each group ranged from 1-7 individuals with an average 60.7% of group members sampled.

3.3.2 Molecular methods

Genomic DNA was isolated from a 2 mm² blood-soaked sample taken from stored cards, using the QIAGEN DNeasy Blood and Tissue kit (QIAGEN Inc., Valencia, CA, USA) as per the protocol of the manufacturer. DNA isolates were quantified using a Qubit® fluorometer kit (Invitrogen). Sex was determined by amplifying the CHD-1 (chromo-helicase-DNA-binding) gene in 20 μl reactions containing 8 μl of Go Taq (Promega), 1 μl of avian sexing primers P2 and P8 (Griffiths et al. 1998) (10 μm) and 2 μl of template. PCR thermal cycles consisted of: 94 °C for 3 min, then 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s,
and a final elongation at 72 °C for 4 min. Sexes were identified from PCR products visualised by gel electrophoresis on E-Gel® SYBR® Safe 2% agarose gels (Invitrogen). DNA isolates were genotyped for 13 Grey-crowned Babbler microsatellite loci: Pte101, Pte102, Pte103, Pte105, Pte106, Pte108, Pte109 (Kawano et al. 2007), Pte17, Pte24, Pte42, Pte47, Pte48, Pte50 (Blackmore et al. 2006) (Table A2.1). The forward primer of each loci was labelled with an appropriate fluorescent tag: FAM (GeneWorks), NED, PET or VIC (Applied Biosystems). Samples were subsequently genotyped by the Australian Genomic Research Facility on an AB3730 capillary sequencer and analysed using Genemapper 3.7 (Applied Biosystems).

### 3.3.3 Microsatellite descriptive statistics

Hardy-Weinberg and linkage equilibrium were assessed using Genepop v 4.2 (Raymond and Rousset 1995) with a Bonferroni correction for multiple comparisons (Rice 1989). Hardy-Weinberg equilibrium and linkage equilibrium conformance tests were conducted for all loci (or locus pair) and across the three regions (west; south-east; north-east). Loci were checked for the presence of null alleles by looking for consistent departures from Hardy-Weinberg equilibrium in the direction of homozygous excess. Genotypic data for loci were manually checked for sex-linkage.

Research on cooperatively-breeding species sometimes remove putative offspring to reduce biasing results from including closely related individuals (Harrisson et al. 2013; Amos et al. 2014). However, following parentage assignments using CERVUS (Kalinowski et al. 2007), we tested each analysis and
compared preliminary results for all individuals with the results of un-related individuals only. No differences between data sets were detected in the results, therefore data included all individuals \((n = 135)\) to provide greater analytical power.

### 3.3.4 Genetic diversity

We used a range of metrics to explore patterns of genetic diversity across the southern range of the Grey-crowned Babbler. GenAlEx v 6.5 (Peakall and Smouse 2006) was used to calculate mean number of alleles \((A)\), mean expected \((H_e)\) and observed \((H_o)\) heterozygosity and the number of private alleles \((P_o;\) alleles unique to the region of sampling) across loci for each of the three regions. Private alleles can provide a simplistic measure of genetic distinctiveness. Allelic richness \((A_r)\) was calculated in FSTAT (Goudet 1995) as a more robust measure of allelic diversity that is standardised for sample size differences.

### 3.3.5 Population genetic structure

Individual-based analyses reflect population processes at finer spatial and temporal scales than population-based analyses (Dutta et al. 2013). To investigate contemporary population structure we used the individual-based Bayesian clustering method in TESS v 2.3 (Durand et al. 2009). TESS implements a spatially explicit algorithm (i.e. uses geographical coordinates of sampling locations) to resolve weaker population structure than comparative non-spatial methods (e.g. STRUCTURE (Chen et al. 2007)) (Durand et al. 2009). TESS was run using the CAR admixture model (100 iterations of \(10^6\) sweeps, discarding the first 30,000), with the spatial interaction parameter set to 0.6 and the number of
genetic clusters (K) set from 2-20. The point of greatest change in a plot of mean DIC across 100 runs against K was used to infer the most likely value of K. Cluster probabilities were averaged for the 10 runs with the lowest DIC values for the most likely K value using the Greedy algorithm option with 1,000 random input orders in CLUMPP v 1.1.2 (Jakobsson and Rosenberg 2007). Results were visualised using DISTRUCT v 1.1 (Rosenberg 2004). For each individual TESS calculates proportional assignment values (Q) to each genetic cluster. We used a threshold of Q ≥0.80 to assign an individual to a particular cluster (labelled 1-4; Fig. 3.1).

3.3.6 Isolation-by-distance and dispersal patterns

To test for isolation-by-distance effects and evidence of sex-biased dispersal at a fine-scale, we compared the spatial genetic structure of all individuals, and for males and females separately, for distances between 0-60 km. We conducted spatial autocorrelation analyses implemented in GenAlEx v 6.5 (Smouse and Peakall 1999) to calculate the spatial autocorrelation coefficient (r) as a measure of mean genetic similarity (relatedness levels) among all pairs of individuals, partitioned into specified geographical distance class bins. Following Peakall and Smouse (2006), we selected distance class bins based on a priori knowledge of Grey-crowned Babbler breeding ecology and territory size and for comparative purposes, distances similar to those used previously investigating isolation-by-distance patterns in the Grey-crowned Babbler (Blackmore and Heinsohn 2008; Blackmore et al. 2011). We also sought to achieve a relatively equal spread of sample sizes across distance classes. The r coefficient value was calculated for all
individuals, and for females and males separately, across nine geographic distance classes with a range of 0.5-60 km. Random permutations were run 999 times to generate upper and lower 95% confidence intervals bounding the null hypothesis of no genetic structure ($r = 0$), and bootstrap resampling was run 999 times to generate 95% confidence intervals around $r$. Correlation coefficient values indicated a positive spatial genetic structure if $r$ values were greater than zero, negative structure if $r$ was less than zero and random levels of genetic similarity if $r$ equalled zero. Permuted values were significant if they were either outside of the 95% confidence interval bounds, or error bars did not intersect zero (Smouse and Peakall 1999). Differences between sexes were significant if their respective error bars did not overlap.

3.4 Results

3.4.1 Landscape structural differences

Landscape extent and pattern differed between east and west regions; in the east tree-cover was more dispersed, which provided a larger total area of land with tree-cover (e.g. corridors, scattered trees, woodland patches). In comparison, the western region had higher levels of aggregated tree-cover prevailing as a small number of large patches ($n = 7$; e.g. conservation reserves) with less dispersed tree-cover across the landscape (Table A2.2).
3.4.2  Microsatellite loci statistics

There were no consistent patterns of linkage disequilibrium between any pairs of loci across regions. Conformance tests across regions showed three loci deviated from Hardy-Weinberg equilibrium in the west region only (Pte50; Pte102; Pte47) (Table A2.1). These deviations likely reflect population specific effects, and could arise from local population substructure (i.e. Wahlund effect). No loci showed consistent linkage disequilibrium or Hardy-Weinberg equilibrium deviations across regions and all loci were retained for analyses. Amplification of the CHD gene identified 74 male and 61 female Grey-crowned Babblers.

3.4.3  Genetic diversity across regions

The mean observed ($H_o$) and expected ($H_e$) heterozygosity for all regions were 0.73 and 0.72 respectively (Table 3.1), and the allelic richness ($A_r$) means were similar among the three regions (west = 8.23; south-east = 7.58; north-east = 8.20) (Table 3.1). A total of 28 private alleles were found amongst all loci. The west region recorded the highest number of private alleles ($n = 14$), and the north-east had similarly high numbers of private alleles ($n = 10$). Conversely, the south-east recorded the least number of private alleles ($n = 4$), indicating gene flow may occur from birds emigrating to the south-east from other regions in the study area.
Table 3.1 Genetic diversity metric averages of the Grey-crowned Babbler within its southern edge-of-range population.

Values are shown for: number of alleles ($A$); Allelic Richness ($A_r$); number of private alleles ($P_a$); observed heterozygosity ($H_o$); expected heterozygosity ($H_e$).

<table>
<thead>
<tr>
<th>Region</th>
<th>Sample size</th>
<th>$A$</th>
<th>$A_r$</th>
<th>$P_a$</th>
<th>$H_o$</th>
<th>$H_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>West</td>
<td>51</td>
<td>8.50</td>
<td>8.23</td>
<td>14</td>
<td>0.69</td>
<td>0.72</td>
</tr>
<tr>
<td>South-east</td>
<td>43</td>
<td>6.50</td>
<td>7.58</td>
<td>4</td>
<td>0.75</td>
<td>0.73</td>
</tr>
<tr>
<td>North-east</td>
<td>41</td>
<td>7.64</td>
<td>8.20</td>
<td>10</td>
<td>0.74</td>
<td>0.72</td>
</tr>
<tr>
<td>Global</td>
<td>135</td>
<td>11.00</td>
<td>8.00</td>
<td>28</td>
<td>0.73</td>
<td>0.72</td>
</tr>
</tbody>
</table>

3.4.4 Population genetic substructure amongst regions

TESS found strong genetic substructure across the study region, with the most parsimonious number of clusters being four (Fig. 3.1). Individuals were assigned to a cluster if $Q \geq 80\%$. Ninety-eight percent of individuals in the west region were assigned to either cluster 3 or 4 (Fig. 3.1; Table 3.2). Individuals in Kerang north and Kerang south were almost exclusively assigned to clusters 3 and 4 respectively, with little evidence of admixture indicating a major barrier to dispersal between the two subpopulations that were located less than 20 km apart. In the south-east region 67% of individuals were assigned to cluster 1 (Table 3.2). Except for one bird, all individuals sampled from Lurg and Violet Town north were assigned to cluster 1, whereas individuals from Violet Town south were admixture (cluster 1 and 4), indicating gene flow between the west and south-east regions.
Table 3.2 Cluster assignment of Grey-crowned Babbler individuals within the study regions: west, south-east and north-east.

Values represent the proportion of individuals assigned to each cluster (1-4) ($Q \geq 0.80$) or admixture. Sample sizes are given in parentheses.

<table>
<thead>
<tr>
<th>Cluster identification numbers</th>
<th>West (51)</th>
<th>South-east (43)</th>
<th>North-east (41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>65</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Admixture</td>
<td>2</td>
<td>33</td>
<td>63</td>
</tr>
</tbody>
</table>

In the north-east region individuals were assigned to clusters 1 (5%), 2 (29%) and 3 (2%), or admixture (63%) (Table 3.2), indicating gene flow between the sampled locations. Pechelba individuals were either assigned to cluster 1, or were admixture where the prominent cluster was 1 with other clusters represented in smaller proportions. Individuals sampled from Chiltern were mostly assigned to cluster 2, whilst individuals sampled from Rutherglen had high admixture with evidence of all four clusters (cluster 2 was prominent), suggesting gene flow between the north-east, south-east and west regions.
The overlay map of Australia and New Guinea shows the sub-specific boundary as a thick black line (Edwards 1993a). The study area is shown as a black rectangle. The main map of the study area shows: study regions (black rectangular outlines); sampling sites (black triangles); tree cover (vegetation cover >2 m in height; grey shading). Below the map the plot depicts the population genetic structure of sampled birds with individuals represented by a vertical shaded bar. Details below the plot represent: family groups (1-39), group sample size (thin vertical line); subpopulation (medium line); region (thick line); genetic structure shading legend (clusters 1-4).

Figure 3.1 The global range of the Grey-crowned Babbler, study region and population genetic structure of P.t.temporalis within the bounds of the study area.

The overlay map of Australia and New Guinea shows the sub-specific boundary as a thick black line (Edwards 1993a). The study area is shown as a black rectangle. The main map of the study area shows: study regions (black rectangular outlines); sampling sites (black triangles); tree cover (vegetation cover >2 m in height; grey shading). Below the map the plot depicts the population genetic structure of sampled birds with individuals represented by a vertical shaded bar. Details below the plot represent: family groups (1-39), group sample size (thin vertical line); subpopulation (medium line); region (thick line); genetic structure shading legend (clusters 1-4).
3.4.5  Isolation-by-distance and dispersal patterns

The mean pairwise genetic similarity (relatedness) between all individuals (Fig. 3.2a) was significantly positive up to a distance of 8 km (i.e. error bars did not intersect zero), and $r$ values decreased as geographic distances increased, indicative of an isolation-by-distance effect. As geographic distances increased from 8 to 60 km, pairwise relatedness of individuals expressed a pattern which would be expected under a random mating scenario until at 60 km, individuals became more dissimilar than expected under random mating (i.e. error bar is below both zero and the lower confidence interval bounds).
Fig. 3.2 Correlogram plots of the spatial genetic autocorrelation coefficient $r$ as a function of distance for (a) all individuals and; (b) males (black line) and females (grey line) separately.

Shown are the upper and lower bounds for the 95% confidence interval about the null hypothesis of no spatial structure ($r = 0$; grey broken lines), and the upper and lower 95% error bars about $r$ as determined by bootstrap resampling.
When sexes were considered separately, an isolation-by-distance effect was observed for females only (Fig. 3.2b). Significant differences between the pairwise relatedness for males and for females (i.e. error bars do not overlap) at distances 2-4 km (Fig. 3.2b) supports our assertion of an isolation-by-distance effect on females. Female Grey-crowned Babbler pairs showed elevated relatedness up to distances of 6-8 km. Between distances of 8-60 km, results indicate female pairwise genetic similarity to be no different from what would be expected under a random mating scenario (i.e. female error bars interact with male error bars, and male error bars cross zero) (Fig. 3.2b). Males on the other hand, did not show elevated levels of relatedness beyond the group-level (<0.5 km) and described a pattern of random genetic similarity for distances >0.5-40 km. Between the two furthest distance classes (40-60 km), males became more genetically dissimilar than expected under random mating, suggesting male gene flow may become restricted beyond 40 km. The presence of isolation-by-distance for females at fine spatial scales and not for males suggests females are driving the overall pattern of isolation-by-distance across the study region and is consistent with a male-biased dispersal pattern.
3.5 Discussion

3.5.1 Influences of geographic distance and barriers on genetic diversity and population structure

Strong population substructure across the southern distribution of the Grey-crowned Babbler was only partially explained by geographic distance (patterns of isolation-by-distance). Strong genetic breaks over short geographic distances observed in the west region (e.g. ~20 km between Kerang north and Kerang south) indicate that specific landscape elements are acting as strong barriers to gene flow. Population substructure can result from various factors including anthropogenic or natural barriers e.g. habitat loss, roads, areas of non-habitat (Sunnucks 2011; Taylor et al. 2011). The genetic break between Kerang north and Kerang south corresponded with a ~5 km radius of agricultural landscapes and roadsides devoid of tree or vegetative cover. Differences in habitat pattern and extent were evident between the west and east regions (Table A2.2). Adjacent to treeless cropping farmscapes, the west region displayed an absence of wooded vegetation along major and minor roads (1,000-2,000 and >2,000 vehicles per day (VPD) respectively) (i.e. Murray Valley Highway; Kerang-Koondrook Road; Kerang-Quambatook Road; Boort-Kerang Road) (Pers. obs.). In contrast, the north-east region and Lurg subpopulation in the south-east indicated gene flow occurrence despite being situated either side of the Hume Highway, a major 4-lane freeway (>5,000 VPD), and which also subdivides Lurg and the two Violet Town subpopulations in the south-east region. The Hume Highway provides high levels of structural habitat connectivity (physical habitat connectedness across
landscapes) from continuous stretches of revegetated roadside habitat and median-strip vegetation in the center of the roadway. In the study area, the Hume Highway is located adjacent to large (>50 ha) conservation reserves, habitat along nearby roadsides, grazing farmscapes with remnant habitat patches, scattered paddock trees and long-term revegetation works (Vesk et al. 2015). It would appear that roadside habitat, including woodland remnants, acts as a conduit for the Grey-crowned Babbler, notwithstanding the threats associated with high VPD levels (e.g. noise disturbance, vehicle strike) (Develey and Stouffer 2001; Parris and Schneider 2009). Higher levels of admixture in the two eastern regions than in the west region was indicative of higher levels of gene flow in the east. Gene flow in the eastern regions could be facilitated by a greater extent of riparian and roadside corridors, coupled with higher levels of dispersed tree cover connecting habitat areas than what was apparent in the west (Fig. 3.1, Table A2.2).

In contrast to abrupt genetic discordance over very short distances in the west, shared cluster membership between the west and north-east regions (cluster 3; Fig. 3.1) provided evidence for gene flow over relatively large geographic distances (~220 km). Grey-crowned Babblers are considered to be a sedentary species with the capacity to cross gaps >300 m within farmland (Robinson 1993; Radford 2008). Fragmented landscapes can necessitate responses such as increased dispersal distances and larger gap-crossing in species that would otherwise avoid such behaviours (Van Houtan et al. 2007). Given the species biology and relatively shorter distances of previous mark-recapture records however, we suggest that observed gene flow at these large spatial scales
is more likely reflecting multi-generational dispersal in a stepping-stone fashion rather than single migration events (Department of the Environment 2015). Gene flow over multiple generations’ at large spatial scales despite high levels of habitat loss and fragmentation is consistent with findings for a suite of other Victorian woodland bird species (Amos et al. 2014). Long-distance movements, however, are often difficult to detect through observational banding studies and only a relatively small number of migrants per generation (e.g. 1-10 per generation (Mills and Allendorf 1996)) are required to homogenise genetic structure (Palsbøll et al. 2007; Allendorf et al. 2008), therefore individual’s dispersal ability may be greater than previously inferred (Robinson 1993; Radford 2008).

The observed long-distance genetic connectivity may be explained by instances of groups’ proximity and available landscape connectivity to major riparian corridors. For example, the majority of individuals assigned to cluster 3 in the west and north-east regions (separated by 220 kilometres) were located within habitat <5 km from continuous riparian corridors encompassing the Murray River or a major tributary, the Goulburn River (Fig. 3.1). Bird species are known to rely on riparian corridors as conduits in fragmented landscapes (Vergara et al. 2013; Volpe et al. 2014). Riparian corridors therefore, may play a role in the functional connectivity for the Grey-crowned Babbler in our study area as has been demonstrated for numerous other species (Cushman et al. 2009).

Genetic diversity levels were similar across the three regions. However, differences in the number of private alleles across the three regions may indicate reduced gene flow among subpopulations. The small number of private alleles in the south-east ($n = 4$) suggests that this region predominantly represents a
3.5.2 **Fine-scale population structure and sex-specific effects of habitat loss and fragmentation**

Fine-scale population genetic structure and an isolation-by-distance effect were evident across our study region. When tested separately, females displayed pairwise significant positive relatedness for distances up to 8 km while males showed no relatedness to each other beyond their groups (>0.5 km). In comparison, significant positive relatedness between individuals of each sex in continuous habitat in the central part of their north-east range (i.e. northern New South Wales) did not extend beyond 1.5 km (Blackmore *et al.* 2011). Therefore, as the observed fine-scale population structure in our study appeared to be driven by elevated relatedness among females, results here could be indicative of a male-biased dispersal pattern in the study area. As this study is the first to describe both a sex-bias dispersal pattern and strong isolation-by-distance effects for females, we suggest such patterns could be explained from high levels of habitat loss and declines in landscape connectivity. Other avian studies have also described restricted mobility across fragmented landscapes leading to adverse sex-specific demographic effects for different species (Dale 2001; Harrisson *et al.* 2012; Amos *et al.* 2014). Alternatively, in contrast with several previous studies suggesting a lack of sex-bias dispersal for the species (King 1980; Eguchi *et al.* 2007; Blackmore *et al.* 2011), females in this area are less dispersive than males.
3.6 Conclusions and management recommendations

Fragmented landscapes are not dichotomies of habitat versus non-habitat, but rather represent a gradient of habitat qualities that may display temporal plasticity (Bennett et al. 2006; Sunnucks 2011). Gene flow was disrupted over distances as little as <20 km in our study landscape, indicating strong barriers to gene flow were present (e.g. treeless areas). Despite strong barrier effects over short distances, we detected evidence of long-distance gene flow (~220 km), which is likely influenced by several factors including an individual’s sex, structural connectivity and/or distance. The strong evidence of isolation-by-distance for females suggests distance is a limiting factor at fine spatial scales for this sex. In contrast, a lack of isolation-by-distance for male Grey-crowned Babblers at distances <40 km could suggest higher levels of landscape permeability for males than females at finer spatial-scales.

Given Grey-crowned Babbler numbers are estimated to be only ~10% of historical levels in the southern parts of its range (Robinson 2006), populations across our study area are likely to be experiencing negative effects of small effective population size (Frankham 1996). Our study suggests that loss of genetic connectivity as a result of habitat loss and fragmentation may have contributed to the Grey-crowned Babbler’s decline. Increasing functional connectivity across the study landscape would be effective in counteracting genetic drift and improving chances of population persistence. Although levels of genetic diversity did not differ across the three study regions, different regions were associated with private alleles. The formation of small isolated populations as a result of
landscape modification can lead to a reduction in the overall genetic diversity of a species (Frankham 1996). Our results identify dispersal barriers and conduits that may be manipulated to help facilitate the movements of the Grey-crowned Babbler across the landscape and provide scope for genetic diversity to be increased within regions by increasing gene flow among regions. Habitat corridors are known to be a critical factor in maintaining genetic exchange (Epps et al. 2007) and we suggest that conservation management plans in fragmented landscapes particularly, need to consider opportunities to increase and enhance corridor areas. Further research that includes investigations into migration levels and individuals’ ancestry can provide important knowledge of spatial and temporal dispersal within the study area and comparisons between contemporary and historical patterns of gene flow will add to our understanding of fragmentation effects on this species. Conservation management plans for southern populations of the species now occupying highly fragmented landscapes, may benefit from comparisons with other populations to determine if they contain unique genetic diversity relative to northern counterparts.
Table 3.3 Authorship statement for Chapter 3.

1. Details of publication and executive author

<table>
<thead>
<tr>
<th>Title of Publication</th>
<th>Publication details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic structure and sex-biased dispersal of a declining cooperative-breeder, the Grey-crowned Babbler (<em>Pomatostomus temporalis</em>), at the southern edge of the species’ range.</td>
<td>Emu – Austral Ornithology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/Institute/Division if based at Deakin; Organisation and address if non-Deakin</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kate Stevens</td>
<td>School of Life and Environmental Sciences</td>
<td><a href="mailto:kpst@deakin.edu.au">kpst@deakin.edu.au</a></td>
</tr>
</tbody>
</table>

2. Inclusion of publication in a thesis

<table>
<thead>
<tr>
<th>Is it intended to include this publication in a higher degree by research (HDR) thesis?</th>
<th>Yes</th>
<th>If Yes, please complete Section 3 If No, go straight to Section 4.</th>
</tr>
</thead>
</table>

3. HDR thesis author’s declaration

<table>
<thead>
<tr>
<th>Name of HDR thesis author if different from above. (If the same, write “as above”)</th>
<th>School/Institute/Division if based at Deakin</th>
<th>Thesis title</th>
</tr>
</thead>
<tbody>
<tr>
<td>As above</td>
<td>School of Life and Environmental Sciences</td>
<td>Conservation of species in fragmented landscapes: Implications for the ecology and biology of the Grey-crowned Babbler (<em>Pomatostomus temporalis</em>)</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

conception of the project, experimental design, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

Signature and date 28 Nov. 2015

4. Description of all author contributions

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katherine A. Harrisson</td>
<td>analysis,</td>
</tr>
<tr>
<td>School of Biological Sciences, Monash University, Melbourne, Victoria, Australia</td>
<td>critical revision of ms for important intellectual content</td>
</tr>
<tr>
<td>Rohan H. Clarke</td>
<td>conception of the project, experimental design, data collection, revision of ms</td>
</tr>
<tr>
<td>School of Biological Sciences, Monash University, Melbourne, Victoria, Australia</td>
<td></td>
</tr>
<tr>
<td>Raylene Cooke</td>
<td>conception of the project, experimental design, revision of ms</td>
</tr>
<tr>
<td>School of Life and Environmental Sciences, Deakin University, Melbourne, Victoria, Australia</td>
<td></td>
</tr>
<tr>
<td>Fiona E. Hogan</td>
<td>conception of the project, data collection, analysis, revising it critically for important intellectual content</td>
</tr>
<tr>
<td>School of Applied and Biomedical Sciences, Federation University Australia, Churchill, Victoria, Australia</td>
<td></td>
</tr>
</tbody>
</table>

5. Author Declarations

I agree to be named as one of the authors of this work, and confirm:

that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,

that there are no other authors according to these criteria,

that the description in Section 4 of my contribution(s) to this publication is accurate,
that the data on which these findings are based are stored as set out in Section 7 below. If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further consent to the incorporation of the publication into the candidate’s HDR thesis submitted to Deakin University and, if the higher degree is awarded, the subsequent publication of the thesis by the university (subject to relevant Copyright provisions).

<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katherine Harrison</td>
<td>02/12/2015</td>
<td></td>
</tr>
<tr>
<td>Rohan Clarke</td>
<td>03/12/2015</td>
<td></td>
</tr>
<tr>
<td>Raylene Cooke</td>
<td>01/12/2015</td>
<td></td>
</tr>
<tr>
<td>Fiona Hogan</td>
<td>3/12/2015</td>
<td></td>
</tr>
</tbody>
</table>

6. Data storage

The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

<table>
<thead>
<tr>
<th>Data format</th>
<th>Storage Location</th>
<th>Date lodged</th>
<th>Name of custodian if other than the executive author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spread sheets</td>
<td>Deakin University: \student-home-\m.its.deakin.edu.au</td>
<td>2011-2015</td>
<td></td>
</tr>
</tbody>
</table>

This form must be retained by the executive author, within the school or institute in which they are based. If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis with the publication.
Gene flow declines for a cooperatively-breeding bird reflect over two centuries of landscape-scale habitat loss and fragmentation.

A pair of breeding-aged Grey-crowned Babblers spreading their wings (and their genes?)
4.1 Abstract

Conservation management relies in part on understanding the effects of landscape modification on gene flow, and knowledge of landscape connectivity levels required for maintaining gene flow and viable population sizes. We used different genetic methods to test whether contemporary levels of gene flow were lower than historical levels among the Grey-crowned Babbler in the southern parts of their range. We analysed genotypic data for 135 individuals to: 1) investigate levels of historical (i.e. pre-fragmentation) and contemporary (i.e. post-fragmentation) gene flow per generation, and the ancestry of individuals, among subpopulations; 2) screen for signatures of bottlenecks and estimate contemporary and historical effective population sizes; 3) explore the relative influences of drift and migration in shaping contemporary population structure, and; 4) provide management recommendations for the Grey-crowned Babbler.

Contemporary (previous 2-3 generations) mean rates of gene flow (i.e. proportion of a subpopulation that are migrants per generation) among the six subpopulations were low (range: 0.01-0.19). Mutation-scaled, long-term mean gene flow rates per generation for a subset of migratory routes were moderate (range: 5.00-11.26). Contemporary levels of effective number of migrants per generation between the east and west regions showed declines of up to 98% from historical levels. Contemporary effective population size estimates for the east and west regions were well below that required for long-term population viability. Demographic history models indicated that genetic drift was a greater influence on subpopulations than gene flow, and five of the six subpopulations showed
signatures of bottlenecks. Results indicate that the functional connectivity of landscapes used by the Grey-crowned Babbler is severely compromised in the study area. The genetic information revealed in this study is critical for informing a strategic conservation intervention program. Knowing the difference in contemporary gene flow compared to historic gene flow has the potential to inform scientifically validated translocation programs that aim to reduce the disparity of gene flow observed between these different time periods. In the long-term, targeted habitat restoration should be utilised to improve the natural permeability of the landscape between subpopulations impacted by habitat fragmentation.

4.2 Introduction

Habitat loss and fragmentation have a major influence on the structure and viability of animal populations in heterogeneous landscapes (Hanski et al. 1995; Villard et al. 1999; Ortego et al. 2015). Landscape-scale anthropogenic habitat modification can lead to isolated subpopulations that are at an increased risk of local patch extinction (Hanski 1998; Fuhlendorf et al. 2002; Banks et al. 2005). Patch occupancy levels are often positively correlated with metapopulation persistence, hence increasing rates of local patch extinctions can foreshadow regional declines (Hanski 1991; Gaston et al. 1997; Elkin and Possingham 2008).

Dispersal of individuals promotes gene flow among habitat patches and is crucial for recolonising suitable vacant habitat patches, maintaining genetic diversity and mitigating extinction risk (Bowler and Benton 2005). The process of
dispersal is influenced by landscape connectivity, this being the degree to which landscapes facilitate the movement of populations, individuals and ultimately genes (Taylor et al. 1993). Landscape connectivity has two components: structural connectivity and functional connectivity. Structural connectivity refers to a landscape’s physical elements and configuration, while functional connectivity refers to an animal’s ability to move through the landscape (Tischendorf and Fahrig 2000), both of which are important for population persistence in modified landscapes.

Gene flow is a fundamental population process and in small, fragmented populations, can play a key role in counteracting the negative effects of inbreeding and genetic drift (Frankham 2005). Understanding the long-term implications of contemporary gene flow rates, and estimates of landscape connectivity levels required to maintain gene flow and viable population sizes, are crucial for conservation of vulnerable populations (Fahrig 2007; Sunnucks 2011). Small and/or declining populations lose genetic diversity through random genetic drift, leaving them vulnerable to the negative effects of inbreeding and reducing their capacity to adapt to environmental change (Saccheri et al. 1998; O’Grady et al. 2006; Pavlacky et al. 2012). Reduced fitness as a result of inbreeding can have negative implications for a species’ reproductive rate, population size and likelihood of long-term population persistence (Keller 1998). In populations with small effective population sizes \( N_e < 100 \); \( N_e \) heuristically, is the number of individuals that can fully contribute to the next generation) it is suggested that over a period of five generations, a population is likely to lose critical functional genes through genetic drift e.g. immunity (Frankham 1995). Although population
sizes equivalent to $N_e > 100$ should limit loss of fitness over five generations to $\leq 10\%$, much larger population sizes (equivalent to $N_e > 1,000$) are required to maintain a population’s ability to adapt to environmental change (Frankham et al. 2014).

Increasing gene flow levels amongst vulnerable and/or declining populations (e.g. via genetic rescue) can reduce inbreeding depression and boost genetic diversity. Greater Prairie Chickens ($Tympanuchus cupido$) have been shown to recover from inbreeding depression as a result of long-term (55-year) conservation efforts that introduced genes from neighbouring populations (Bouzat et al. 1998a; Bouzat et al. 1998b). It is important then to maintain larger populations and enhance their functional connectivity with other smaller and/or isolated populations (i.e. maintain metapopulation processes). More stable and genetically robust populations will promote species persistence and their evolutionary potential under increasing human-induced pressures from landscape modification, extreme events and climate change uncertainties (Nimmo et al. 2015).
4.2.1 Genetic implications for a species experiencing spatial and genetic population structure: the case of the Grey-crowned Babbler

The Grey-crowned Babbler (*Pomatostomus temporalis*) is a woodland bird species adversely affected by human-induced reductions in landscape connectivity (Adam and Robinson 1996; Blackmore *et al.* 2011; Stevens *et al.* in press; Chapter 3). Grey-crowned Babblers are a cooperatively-breeding species, with offspring delaying dispersal from natal territories for up to three years. Local neighbourhoods are characterised by closely-related individuals, suggesting most dispersal occurs over relatively short distances (Koenig *et al.* 1992; Blackmore *et al.* 2011; Stevens *et al.* in press; Chapter 3). Groups occupy territories between 2 and 53 ha (Higgins and Peter 2003; Blackmore and Heinsohn 2008). The range of the eastern subspecies *Pomatostomus temporalis temporalis* in Australia, incorporates approximately two-thirds of eastern Australia (Fig. 4.1). The Grey-crowned Babbler was historically common throughout much of eastern Australia (Department of Environment and Heritage 2013), but has undergone a major range contraction and population declines of over 90% across the southern extent of its distribution as a consequence of habitat loss and fragmentation (Robinson 1993; Environment Conservation Council 1997; Robinson 2006; Environment Australia 2011). In southern parts of its range, the Grey-crowned Babbler is restricted to roadside or riparian vegetation, small adjacent remnant woodland patches within farmland (<0.5 ha) and habitat edges of the few remaining larger conservation reserves (>5 ha) (Robinson 2006).
Figure 4.1 The global distribution of the Grey-crowned Babbler, and the study region and sample site locations of the subspecies, *P. t. temporalis* in its southernmost range.

The overlay map of Australia and New Guinea shows the species global range. The sub-specific boundary is indicated by a thick black line (Edwards 1993a) and the study area is indicated by the black rectangle. The main map of the study area shows: regions (black rectangular outlines); sampling sites (black triangles) that are associated with subpopulations (labels); tree cover (vegetation cover >2 m in height) (grey shading).
Recent avian studies have described landscape fragmentation effects on the dispersal patterns of different species (Coulon et al. 2012; Berkman et al. 2013; Amos et al. 2014) showing that landscape effects can vary amongst species. Stevens et al. (in press; Chapter 3) explored patterns of dispersal and genetic connectivity across the southern parts of the range of the Grey-crowned Babbler, finding less population genetic structure and higher levels of admixture among subpopulations in the eastern regions compared to the western region. Genetic barriers were identified at relatively small spatial scales (<20 km) in the east and west regions, coinciding with areas devoid of tree cover. In contrast, gene flow over much greater distances (up to 270 km) appeared to be facilitated by riparian and roadside habitat corridors (Stevens et al. in press; Chapter 3). Understanding the role of landscape connectivity amongst spatially structured and declining populations is paramount for informing effective long-term conservation measures to promote genetic variation and population demographic viability (Amos et al. 2014).

We investigated the effects of landscape-scale habitat loss and fragmentation on spatial patterns of gene flow in the threatened Grey-crowned Babbler. Here we analysed genotypic data of the Grey-crowned Babbler to: 1) investigate the levels of historical (i.e. pre-fragmentation) and contemporary (i.e. post-fragmentation) gene flow per generation, and the ancestry of individuals, among subpopulations; 2) screen for signatures of genetic bottlenecks and estimate contemporary and historical effective population sizes; 3) explore the relative influences of drift and migration in shaping contemporary population structure, and; 4) provide management recommendations for the Grey-crowned
4.3 Methods

4.3.1 Sampling

This study builds on long-term (~30-yr) surveys and habitat requirement research of the Grey-crowned Babbler in central and north-eastern Victoria (Robinson 1993; Tzaros 1995; Tzaros 2001; Davidson 2009; Lacey, unpub. data; Robinson, unpub.data) by investigating the genetic implications of major population declines as a result of habitat loss and fragmentation. The study region encompassed an area of ~22, 250 km² in north-central and north-east Victoria, Australia (Fig. 4.1).

Call playback confirmed the presence and size of a Grey-crowned Babbler family group at each potential site. Territory occupancy was verified from nesting activity and site locations were recorded using a Geographic Positioning System (GPS). An on-ground search using call playback was conducted in areas of habitat within a 2 km radius of each study territory to determine distance to adjacent groups. An average distance of 979.8 m separated sampled groups and their closest neighbouring Grey-crowned Babbler group, measured between group centroids (usually a nest). DNA sampling was undertaken at 40 sites selected from three geographic regions: west (n = 15, Kerang; Boort), south-east (n = 12, Violet Town; Lurg) and north-east (n = 13, Peechelba; Rutherglen; Chiltern) (Fig. 4.1). The sampling period incorporated two annual breeding seasons between June 2010-April 2012. Birds were lured with call playback and trapped using mist-
nets. Each individual was banded with a metal leg band provided by the
Australian Bird and Bat Banding Scheme (ABBB5) and a unique combination of
tree coloured plastic leg bands for identification in the field. Individuals were
weighed and measured and a blood sample (≈70 μl) collected from the brachial
vein using a VITREX® capillary tube. Blood was transferred to a Whatman FTA
Card® and stored at room temperature in paper envelopes. We sampled 135
Grey-crowned Babbler individuals from 40 sites, each sampling site representing a
discrete family group.

4.3.2 Molecular methods

Genomic DNA was isolated from a 2 mm² blood-soaked sample taken from
stored cards, using the QIAGEN DNeasy Blood and Tissue kit (QIAGEN Inc.,
Valencia, CA, USA) as per the protocol of the manufacturer. DNA isolates were
quantified using a Qubit® fluorometer kit (Invitrogen). Sex was determined by
amplifying the CHD-1 (chromo-helicase-DNA-binding) gene in 20 μl reactions
containing 8 μl of Go Taq (Promega), 1 μl of sex identification markers P2 and P8
(Griffiths et al. 1998) (10 μm) and 2 μl of template. PCR cycles consisted of: 94 °C
for 3 min, then 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final
elongation at 72 °C for 4 min. Sexes were identified from PCR products visualised
by gel electrophoresis on E-Gel® SYBR® Safe 2% agarose gels (Invitrogen). DNA
isolates were genotyped for 13 Grey-crowned Babbler microsatellite loci: Pte101,
Pte102, Pte103, Pte105, Pte106, Pte108, Pte109 (Kawano et al. 2007), Pte17,
Pte24, Pte42, Pte47, Pte48, Pte50 (Blackmore et al. 2006). The forward primer of
each loci was labelled with an appropriate fluorescent tag: FAM (GeneWorks),
NED, PET or VIC (Applied Biosystems). Samples were subsequently genotyped by the Australian Genomic Research Facility on an AB3730 capillary sequencer and analysed using Genemapper 3.7 (Applied Biosystems).

4.3.3 Microsatellite performance, heterozygosity and genetic diversity

Subpopulations were identified within each study region ($n = 6$) based on population genetic substructure identified using a Bayesian cluster assignment method (i.e. TESS (Chen et al. 2007)) (Stevens et al. in press; Chapter 3). The six subpopulations were: 1. Kerang north; 2. Kerang south and Boort; 3. Violet Town south; 4. Lurg, Violet Town north and Peechelba; 5. Rutherglen, and; 6. Chiltern (Fig.4.1).

Microsatellite performance was assessed for all loci using Hardy-Weinberg and linkage equilibrium and performed in Genepop v 4.2 (Raymond and Rousset 1995) with a Bonferroni correction for multiple comparisons (Rice 1989). Loci were checked for the presence of null alleles by looking for consistent departures from Hardy-Weinberg equilibrium (HWE) in the direction of homozygous excess. Genotypic data for loci were manually checked for sex-linkage.

Standard genetic diversity index computations were tested on the six subpopulations. Genetic diversity metrics were estimated for the observed ($H_o$) and expected ($H_e$) heterozygosity and number of private alleles ($P_o$) using GenAlEx v 6.5 (Peakall and Smouse 2006). Allelic richness ($A_r$) was investigated with FSTAT (Goudet 1995) as a more robust measure of allelic diversity that is standardized for sample size differences.
4.3.4  Contemporary gene flow among subpopulations

We investigated contemporary (previous 2-3 generations) gene flow levels using BAYESASS v 3.1.1 (Wilson and Rannala 2003) amongst all six subpopulations (n = 36 possible pairwise comparisons between subpopulations). As the average reproductive lifespan of the Grey-crowned Babbler is 5 years and they exhibit overlapping generations (Counsilman and King 1977), we presumed contemporary gene flow levels to represent the 10-15 years prior to sampling and therefore likely to reflect genetic processes following extensive habitat fragmentation in the study area (Bradshaw 2012).

BAYESASS uses a Bayesian method with Markov chain Monte Carlo (MCMC) and assumes linkage equilibrium, negligible genetic drift over the previous 2-3 generations and relaxes assumptions of HWE within populations (Wilson and Rannala 2003). Markov chain Monte Carlo mixing parameter values for gene flow rates, allele frequencies and inbreeding coefficients were adjusted to 0.50, 0.95 and 0.50 respectively, to achieve recommended acceptance rates (Wilson and Rannala 2003). We performed $3 \times 10^7$ MCMC iterations with $10^6$ iterations to discard as burn-in. Each run was initialised with different starting-seed values to achieve consistency of mean parameter estimates between runs.

We also identified putative first-generation migrants and their inferred origins using BAYESASS. We further sought to validate BAYESASS assignments by implementing the Bayesian approach of Rannala and Mountain (1997) with a Monte Carlo resampling algorithm (Paetkau et al. 2004) in GENECLASS 2 (Piry et al. 2004). We tested 10,000 simulated individuals with a Type I error threshold of 0.05 and used a likelihood ratio $L_{\text{home}}/L_{\text{max}}$. This ratio is computed from the
likelihood of the population from which the individual was sampled ($L_{\text{home}}$) over
the highest likelihood value among all population samples ($L_{\text{max}}$), including the
population of the individual (Piry et al. 2004). The likelihood ratio of $L_{\text{home}}/L_{\text{max}}$ has
more statistical power than using only $L_{\text{home}}$ to identify non-resident individuals
among populations (Piry et al. 2004). Both assignment methods assume all
possible source populations have been sampled. Although some disparate Grey-
crowned Babbler groups exist between our populations (Robinson unpub. data),
analysis allowed us to identify general pathways of dispersal and to make direct
comparisons of ancestry assignments between both methods.

4.3.5 Long-term gene flow estimation

Estimates of long-term gene flow rates and effective population sizes
required a Bayesian approach based on coalescent theory implemented in
MIGRATE v 3.6.5 (Beerli and Felsenstein 2001; Beerli 2006). MIGRATE calculates
the mutation-scaled, long-term effective population size ($\theta = 4N_e\mu$) for each
population and the mutation-scaled, long-term gene flow rates ($M$; defined as
$m/m\mu$, where $m$ is the immigration rate and $\mu$ is the mutation rate per generation)
between population pairs based on the coalescent, and with an $n$-island model in
equilibrium (Beerli and Felsenstein 1999; 2001). MIGRATE differs from traditional
estimates based on $F$-statistics by allowing for unequal population sizes and
asymmetric gene flow, thereby better reflecting biological reality in ecological
systems (Palstra et al. 2007).

We used MIGRATE to estimate mutation-scaled, long-term effective
population size for each of the six subpopulations and gene flow rates among
subpopulations. To reduce the number of potential parameters relative to the number of loci and improve statistical power (Kuhner 2009), we set parameters to include symmetric gene flow and estimated gene flow between geographically adjacent populations rather than all possible pairs of populations, thereby providing a subset of migratory routes \( n = 12 \). We used the Brownian motion model with \( F_{st} \) calculations of \( \theta \) and \( M \) as starting parameters. We used Metropolis-Hastings sampling and uniform prior distributions to estimate \( \theta \) (range: 0-100, delta: 10) and \( M \) (range: 0-500, delta: 50). The Markov chain settings recorded \( 10^4 \) steps from 1 long chain of \( 10^6 \) sampled steps, and a search strategy following a ‘static’ heating scheme using four temperatures (1.0, 1.5, 3.0, 1,000.0) to examine the genealogical space more effectively (Beerli 2009). Runs were replicated twice to ensure posterior probabilities stabilized.

4.3.6 Temporal effective number of migrants per generation

To enable direct comparison between long-term and contemporary gene flow rates, we translated gene flow estimates from BAYESASS and MIGRATE into effective number of migrants per generation \( (Nm) \). To increase the statistical power of our data and reliability of the results, we combined our data into two regions based on the geographical location of samples. This approach provided two large data sets from regions that were identified as ‘west’: 1. Kerang north; Kerang south/Boort \( (n = 51) \) and ‘east’: 2. Violet Town south; Lurg/Violet Town north/Peechelba; Rutherglen; Chiltern \( (n = 84) \) (Fig.4.1). Contemporary gene flow rates were estimated for east and west regions using BAYESASS v 3.1.1 (Wilson and Rannala 2003) with MCMC parameters set at 0.1 for gene flow rates, allele
frequencies and inbreeding coefficients. The resulting gene flow rates were multiplied with the appropriate contemporary effective population sizes ($N_e$) estimated using LDNe (see section 4.3.7 below). Mutation-scaled, long-term migration rates and effective population size were estimated for the two regions in MIGRATE v 3.6.5 (Beerli and Felsenstein 2001; Beerli 2006) and these results were used to calculate the long-term effective number of migrants per generation using the formula ($\theta$*$M$)/4 (specific to nuclear data) (Beerli 2009).

4.3.7 Temporal effective population sizes

Estimates of contemporary effective population size ($N_e$) were calculated by testing for linkage disequilibrium using LDNe (Waples 2008). LDNe uses a single-sample method rather than traditional $N_e$ estimates which require temporally-spaced samples taken from the same population (Nei and Tajima 1981; Waples 1989; Wang and Whitlock 2003). LDNe estimates contemporary $N_e$ based on evidence of linkage disequilibrium among alleles at different loci as a result of drift. The theory presumes linkage disequilibrium will increase as a result of genetic drift to produce non-random associations between un-linked loci more frequently in small rather than large populations (Frankham 1995). A recent study suggests that LDNe analysis can be affected by sample sizes less than 30 (Tallmon et al. 2010). Four of the six subpopulation sample sizes were low (i.e. <30) (Table 4.1). Hence, we estimated effective population sizes for the two regions, east and west, and thereby enabled greater statistical power and reliable estimates for contemporary estimates of $N_e$. We estimated $N_e$ using three different rates for the inclusion of rare alleles ($p_{crit}$: 0.05; 0.02; 0.01), which allowed for
comparisons of consistency across results. We report estimates from the
criterion $\geq 0.05$ as these provide a reasonable balance between maximum
precision and minimal bias with polymorphic loci such as microsatellites (Waples
2008).

Although direct comparison of effective population sizes between the two
time periods were not possible (mutation rate of the species remains unknown),
we investigated if similar patterns of effective population sizes were evident over
time for the two regions. We used MIGRATE to estimate mutation-scaled, long-
term effective population size for the east and west regions. We set similar
parameters as those used for estimates of historic gene flow between the
subpopulations (see previous section 4.3.5), which included symmetric gene flow
and the Brownian motion model with $F_{st}$ calculations of $\theta$ and $M$ as starting
parameters. The search strategy implemented the ‘static’ heating scheme using
four temperatures (1.0; 1.5; 3.0; 1,000.0). Run times were faster than earlier
MIGRATE analyses (section 4.3.5) owing to the reduced number of parameters,
therefore runs were replicated five times.

4.3.8 **Modelling population history**

The genealogical history of the six subpopulations was investigated to
estimate whether drift was more important than immigration in shaping
contemporary population structure (Ciofi et al. 1999). Two models of population
history, drift versus immigration-drift equilibrium (gene flow), were assessed in 2-
MOD v 0.2 following the methods of Ciofi et al. (1999). Both models are based on
allele frequencies in populations. The drift model computes allele frequencies as
a product of pure drift with little evidence of gene flow between populations. The
gene flow model works on an equilibrium principle between immigration and
genetic drift to evaluate allele frequency within populations. The likelihood of
each models’ fit to the data is estimated using MCMC methods which compares
estimates between models and provides probabilities of the goodness of fit for
each (Ciofi et al. 1999). Simulations of MCMC were run for $10^5$ repeats, discarding
the initial 10% of results to avoid possible bias from start conditions, and the
analysis was repeated three times to validate results.

4.3.9 **Signature of bottlenecks within subpopulations**

To investigate whether the six subpopulations had experienced bottlenecks
we implemented two methods in BOTTLENECK v 1.2.02 (Cornuet and Luikart
1996). The first method investigated if observed heterozygosity within each
subpopulation was higher than would be expected for populations in mutation-
drift equilibrium. This test can be used to detect bottlenecks over the last 2-4 $N_e$
generations (potentially hundreds of years prior to sampling) and uses a one-
tailed Wilcoxon test for statistical support (Cornuet and Luikart 1996; Luikart et al.
1998). We ran three different models to investigate excess heterozygosity; the
infinite allele model (IAM), step-wise mutation model (SMM), and a combination
of these in a two-phase mutation model (TPM). The proportion of step-wise
mutation model in the two-phase mutation model was set to 70%. Our second
approach implemented the mode-shift method in BOTTLENECK which tests for
more recent occurrences of bottlenecks ($\leq 12$ previous generations) within the six
subpopulations. This approach works on the premise that populations’
experiencing recent bottlenecks will demonstrate a smaller proportion of alleles at lower frequencies (<10%) than intermediate frequencies (L-shaped shifted mode distribution) as alleles at low frequencies are more likely to be lost during a bottleneck (Cornuet and Luikart 1996; Luikart et al. 1998). Using both approaches enabled us to test for bottleneck occurrences among different temporal generations before and after extensive habitat fragmentation in the study area (Bradshaw 2012).

4.4 Results

4.4.1 Genetic diversity amongst subpopulations

All loci were shown to behave according to Hardy-Weinberg equilibrium and linkage equilibrium. Across all loci, mean $H_o$ and $H_e$ heterozygosity’s was 0.72 and 0.68 respectively. All six subpopulations had private alleles providing a total of 28 private alleles across the study population. Rutherglen, Kerang north and Kerang south/Boort had the highest number of private alleles (8, 7 and 7 respectively) suggesting that these subpopulations have been isolated at some recent point in time. The three remaining subpopulations (Lurg/Violet Town north/Peechelba; Violet Town south; Chiltern) all had two private alleles each (Table 4.1). Allelic richness means were similar among subpopulations, and ranged from 4.69 (Violet Town south) to 5.65 (Rutherglen) (Table 4.1).
Table 4.1 Genetic diversity of the Grey-crowned Babbler within six subpopulations at their southern range limit.

Values show: number of samples \((n)\), observed heterozygosity \((H_o)\), expected heterozygosity \((H_e)\), number of private alleles \((P_a)\) and mean allelic richness \((A_r)\). Subpopulations are: Kerang north (Kn); Kerang south/Boort (KSB); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Rutherglen (Rg); Chiltern (Ch).

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>(n)</th>
<th>(H_o)</th>
<th>(H_e)</th>
<th>(P_a)</th>
<th>(A_r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kn</td>
<td>13</td>
<td>0.670</td>
<td>0.603</td>
<td>7</td>
<td>5.048</td>
</tr>
<tr>
<td>KSB</td>
<td>38</td>
<td>0.700</td>
<td>0.704</td>
<td>7</td>
<td>5.624</td>
</tr>
<tr>
<td>Vs</td>
<td>10</td>
<td>0.723</td>
<td>0.660</td>
<td>2</td>
<td>4.692</td>
</tr>
<tr>
<td>LVP</td>
<td>37</td>
<td>0.754</td>
<td>0.707</td>
<td>2</td>
<td>5.533</td>
</tr>
<tr>
<td>Rg</td>
<td>21</td>
<td>0.739</td>
<td>0.678</td>
<td>8</td>
<td>5.653</td>
</tr>
<tr>
<td>Ch</td>
<td>16</td>
<td>0.740</td>
<td>0.651</td>
<td>2</td>
<td>5.075</td>
</tr>
<tr>
<td>All individuals</td>
<td>135</td>
<td>0.721</td>
<td>0.667</td>
<td>28</td>
<td>5.271</td>
</tr>
</tbody>
</table>

4.4.2 Contemporary gene flow rates and ancestry

Contemporary gene flow rates per generation amongst all six subpopulations were low and ranged from 0.01-0.19 (Table 4.2). The largest gene flow rate, whilst low, was observed between Kerang south/Boort to Violet Town south (0.19) and represented a west to east direction of gene flow, and was a surprising result as the two subpopulations are geographically separated by ~170 km. Contemporary gene flow rates between the remaining 35 possible pairwise migratory routes ranged from 0.01-0.07. Two subpopulations showed similar rates of bidirectional gene flow (Chiltern to Rutherglen, 0.07; Rutherglen to Chiltern, 0.05) (Table 4.2).
Amongst all subpopulations, Kerang south/Boort had the lowest contemporary net immigration rate per generation (sum of incoming gene flow minus the sum of outgoing gene flow; -0.25) Violet Town south had the highest net immigration rate (0.25) indicating the subpopulation may be receiving more immigrants per generation than emigrants.

Individual ancestry assignments computed with BAYESASS identified 10 likely first-generation migrants (Table 4.3). The ancestry probability of all first-generation birds was 0.87. Amongst adult (≥2nd year) migrants, a male-bias was evident (males, n = 7; females, n = 1). One immature (1st year bird) female and one immature male were identified as first-generation migrants. Ancestry assignments using GENECLASS 2 differed from BAYESASS and identified nine individuals as first-generation migrants (p < 0.05), and showed a similar male-bias pattern amongst adult migrants (males, n = 6; females, n = 1) (Table 4.3). Three individuals identified in GENECLASS 2 were also identified as first-generation migrants in BAYESASS, including the two immature birds.

Given we did not exhaustively sample all possible source populations, the assigned populations of origin of first generation migrants should be treated with a degree of caution. We conservatively presumed the most likely first-generation migrants from our results were the three individuals (2 immature; 1 adult) identified in both methods as first-generation migrants (Table 4.3). The mean posterior probability (BAYESASS) and mean log likelihood (GENECLASS 2) ancestry for these three migrants was 0.72 and 17.89 respectively. Euclidean and structural connectivity distances between the sampling location and place of origin differed between age classes of the three birds (immature: <20 km, <30 km;
adult: ~220 km, ~280 km respectively). The three migrants were sampled from
three subpopulations (Violet Town south, Chiltern and Rutherglen), and putative
ancestry origins also identified three subpopulations (Lurg, Rutherglen and Kerang
south/Boort; ordered correspondingly) (Table 4.3).

4.4.3 Long-term gene flow rates

The overall MIGRATE mean mutation-scaled, long-term rates of gene flow
(M) per generation between the subset of migratory routes (n = 12) was 7.08 and
ranged from 5.00 (bidirectional gene flow between Lurg/Violet Town
north/Peechelba and Violet Town south) to 11.26 (bidirectional gene flow
between Chiltern and Rutherglen).

4.4.4 Long-term and contemporary effective number of migrants per
generation

The contemporary effective number of migrants per generation (Nm) for
the east and west regions indicated a major decline in gene flow between the two
regions over time. Both regions showed similar high proportions of gene flow
decline (west = 98%; east = 94%) from historical levels. This result suggests that
while contemporary gene flow still occurs at very low levels from the west to the
east, even fewer numbers of migrants are successfully dispersing in the reverse
direction.
Table 4.2 Estimates of recent (previous 2-3 generations) mean gene flow rates per generation among six Grey-crowned Babbler subpopulations.

Values indicate the mean proportion of individuals within subpopulations in rows (‘Destination of gene flow’) that are immigrants from subpopulations in columns (‘Origins of gene flow’). Gene flow rates are presented on the first line of a cell and 95% confidence intervals in parentheses are on a cell’s second line. Proportions of non-migrants are on the diagonal and in bold type. The highest recorded gene flow is indicated with an asterisk (*). Subpopulations are: Kerang north (Kn); Kerang south/Boort (KSB); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Rutherglen (Rg); Chiltern (Ch). Values were calculated using BAYESASS (Wilson and Rannala 2003).

<table>
<thead>
<tr>
<th>Destination of gene flow</th>
<th>Kn</th>
<th>KSB</th>
<th>Vs</th>
<th>LVP</th>
<th>Rg</th>
<th>Ch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kn</td>
<td>0.91</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(-0.04-0.10)</td>
<td>(-0.03-0.03)</td>
<td>(-0.03-0.03)</td>
<td>(-0.03-0.03)</td>
<td>(-0.04-0.04)</td>
<td>(-0.03-0.03)</td>
</tr>
<tr>
<td>KSB</td>
<td>0.01</td>
<td>0.96</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>(-0.02-0.02)</td>
<td>(-0.02-0.05)</td>
<td>(-0.01-0.01)</td>
<td>(-0.02-0.02)</td>
<td>(-0.02-0.02)</td>
<td>(-0.02-0.02)</td>
</tr>
<tr>
<td>Vs</td>
<td>0.03</td>
<td>0.19*</td>
<td>0.69</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(-0.05-0.05)</td>
<td>(-0.07-0.09)</td>
<td>(-0.03-0.06)</td>
<td>(-0.06-0.06)</td>
<td>(-0.04-0.04)</td>
<td>(-0.05-0.05)</td>
</tr>
<tr>
<td>LVP</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.94</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>(-0.02-0.02)</td>
<td>(-0.03-0.03)</td>
<td>(-0.01-0.04)</td>
<td>(-0.02-0.07)</td>
<td>(-0.02-0.02)</td>
<td>(-0.01-0.01)</td>
</tr>
<tr>
<td>Rg</td>
<td>0.03</td>
<td>0.04</td>
<td>0.01</td>
<td>0.02</td>
<td>0.83</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(-0.04-0.04)</td>
<td>(-0.05-0.05)</td>
<td>(-0.02-0.03)</td>
<td>(-0.03-0.03)</td>
<td>(-0.04-0.11)</td>
<td>(-0.06-0.06)</td>
</tr>
<tr>
<td>Ch</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>(-0.03-0.03)</td>
<td>(-0.04-0.04)</td>
<td>(-0.03-0.03)</td>
<td>(-0.04-0.04)</td>
<td>(-0.05-0.05)</td>
<td>(-0.04-0.10)</td>
</tr>
</tbody>
</table>
Table 4.3 First-generation migrants identified among six genetic subpopulations of the Grey-crowned Babbler in southern parts of its range. Values indicate the probability (BAYESASS) and/or the log likelihood (log(L)) (GENECLASS), of an individual being a first-generation migrant. Euclidean distances are approximations and are measured from the sampling location of the immigrant to the nearest family group from the putative subpopulation of origin. Subpopulations are: Chiltern (Ch); Rutherglen (Rg); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Kerang south/Boort (KSB); Kerang north (Kn). All GENECLASS probability values were below the significance threshold (i.e. $p < 0.05$). Results are shown in descending order based on probability, then log(L), values.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Sample location</th>
<th>Origin of ancestry</th>
<th>Probability of ancestry (BAYESASS)</th>
<th>log(L) of ancestry (GENECLASS)</th>
<th>Sex</th>
<th>Age class</th>
<th>Approximate Euclidean distance (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VB085ᵃ</td>
<td>Vs</td>
<td>KSB</td>
<td>0.979</td>
<td></td>
<td>male</td>
<td>Adult</td>
<td>170</td>
</tr>
<tr>
<td>VB086ᵃ</td>
<td>Vs</td>
<td>KSB</td>
<td>0.956</td>
<td></td>
<td>male</td>
<td>Adult</td>
<td>170</td>
</tr>
<tr>
<td>VA135ᵃ</td>
<td>Vs</td>
<td>KSB</td>
<td>0.950</td>
<td></td>
<td>male</td>
<td>Adult</td>
<td>175</td>
</tr>
<tr>
<td>VB087ᵃ</td>
<td>Vs</td>
<td>KSB</td>
<td>0.946</td>
<td></td>
<td>male</td>
<td>Adult</td>
<td>170</td>
</tr>
<tr>
<td>VB088ᵃ</td>
<td>Vs</td>
<td>KSB</td>
<td>0.934</td>
<td></td>
<td>male</td>
<td>Adult</td>
<td>170</td>
</tr>
<tr>
<td>VA092ᵃ</td>
<td>Vs</td>
<td>KSB</td>
<td>0.910</td>
<td></td>
<td>male</td>
<td>Adult</td>
<td>175</td>
</tr>
<tr>
<td>VA134ᵃ</td>
<td>Vs</td>
<td>KSB</td>
<td>0.892</td>
<td></td>
<td>female</td>
<td>Adult</td>
<td>175</td>
</tr>
<tr>
<td>CH006ᵇ</td>
<td>Ch</td>
<td>Rg</td>
<td>0.872</td>
<td>16.804</td>
<td>male</td>
<td>Immature</td>
<td>15</td>
</tr>
<tr>
<td>VA091ᵇ</td>
<td>Vs</td>
<td>LVP</td>
<td>0.794</td>
<td>19.156</td>
<td>female</td>
<td>Immature</td>
<td>20</td>
</tr>
<tr>
<td>RH035ᵇ</td>
<td>Rg</td>
<td>KSB</td>
<td>0.485</td>
<td>17.706</td>
<td>male</td>
<td>Adult</td>
<td>220</td>
</tr>
<tr>
<td>RI008ᵇ</td>
<td>Ch</td>
<td>Rg</td>
<td>0.485</td>
<td>14.204</td>
<td>male</td>
<td>Adult</td>
<td>12</td>
</tr>
<tr>
<td>VT068ᵇ</td>
<td>KSB</td>
<td>LVP</td>
<td>0.485</td>
<td>16.254</td>
<td>male</td>
<td>Adult</td>
<td>190</td>
</tr>
<tr>
<td>CH004ᵇ</td>
<td>Rg</td>
<td>Ch</td>
<td>0.485</td>
<td>18.517</td>
<td>male</td>
<td>Adult</td>
<td>15</td>
</tr>
<tr>
<td>RR040ᵇ</td>
<td>LVP</td>
<td>Rg</td>
<td>0.485</td>
<td>20.036</td>
<td>female</td>
<td>Adult</td>
<td>12</td>
</tr>
<tr>
<td>RE131ᵇ</td>
<td>Kn</td>
<td>Rg</td>
<td>0.485</td>
<td>20.761</td>
<td>male</td>
<td>Adult</td>
<td>215</td>
</tr>
<tr>
<td>CK042ᵇ</td>
<td>LVP</td>
<td>Ch</td>
<td>0.485</td>
<td>21.958</td>
<td>male</td>
<td>Adult</td>
<td>37</td>
</tr>
</tbody>
</table>

ᵃ Individual identified using BAYESASS; ᵇ individual identified using GENECLASS 2
4.4.5 Long-term and contemporary effective population sizes

MIGRATE estimates of mutation-scaled, long-term effective population sizes ($\theta$) estimated the east (5.89) was higher than the west (4.29) (Table 4.4). LDNe estimates of the contemporary effective population sizes ($N_e$) for the east and west regions showed both regions’ effective population size were smaller than their respective sample size ($n$) (east: $n = 83, N_e = 19.7$; west $n = 51, N_e = 17.0$). Although direct comparison of the values for effective number population sizes were not possible between the two time periods, highest and lowest values for each time period were estimated for the same regions (i.e. lowest = west; highest = east) (Table 4.4).

Table 4.4 Estimates of long-term and contemporary effective number of migrants per generation and effective population sizes for two geographically separated regions of Grey-crowned Babbler in the southern part of their distribution.

<table>
<thead>
<tr>
<th>Migrant to</th>
<th>$n$</th>
<th>Long-term $Nm$</th>
<th>Contemporary $Nm$</th>
<th>Temporal $Nm$ difference</th>
<th>$\theta$</th>
<th>$N_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>west</td>
<td>51</td>
<td>16.52</td>
<td>0.31</td>
<td>16.21</td>
<td>4.29</td>
<td>17.0</td>
</tr>
<tr>
<td>east</td>
<td>84</td>
<td>22.68</td>
<td>1.35</td>
<td>21.33</td>
<td>5.89</td>
<td>19.7</td>
</tr>
</tbody>
</table>
4.4.6 **Demographic history of subpopulations**

Comparisons between the likelihood of models to best explain the genetic history of subpopulations revealed all replicate runs to select the pure drift model over the gene flow model (probability: drift = 0.70; gene flow = 0.30). This result suggests low levels of gene flow among subpopulations that may not be sufficient to counterbalance genetic drift.

4.4.7 **Bottleneck signatures within subpopulations**

The heterozygosity excess test for longer-term bottleneck signatures provided significant results ($p < 0.05$) indicating different supporting models amongst the six subpopulations (Table 4.5). Four of the six subpopulations (Kerang south/Boort, Violet Town south, Lurg/Violet Town north/Peechelba and Rutherglen) were supported by two or more models that included the two-phase mutation model (the most appropriate model for microsatellite data (Di Rienzo et al. 1994)). Chiltern was supported only by the infinite allele model and Kerang north was not supported by any of the three models, suggesting that this subpopulation was in mutation-drift equilibrium (Table 4.5).

The tests for more recent bottleneck occurrences among subpopulations (i.e. \( \leq 60 \) years) showed Lurg/Violet Town north/Peechelba as the only subpopulation having evidence of a ‘mode-shift’ in allele frequencies (results not shown) and suggests the occurrence of bottlenecks in recent generations.
Table 4.5 Three models showing Bottleneck signature values for Grey-crowned Babbler individuals from six subpopulations.

Values show: mean number of samples ($N$); mean observed number of alleles ($k$). Significant values ($p < 0.05$) for; infinite allele model (IAM); two-phase mutation model (TPM); step-wise mutation model (SMM). Subpopulations are: Kerang north (Kn); Kerang south/Boort (KSB); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Rutherglen (Rg); Chiltern (Ch).

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>$N$</th>
<th>$k$</th>
<th>IAM ($p$)</th>
<th>TPM ($p$)</th>
<th>SMM ($p$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kn</td>
<td>25.85</td>
<td>5.46</td>
<td>0.19</td>
<td>0.66</td>
<td>0.96</td>
</tr>
<tr>
<td>KSB</td>
<td>76.00</td>
<td>7.23</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>0.61</td>
</tr>
<tr>
<td>Vs</td>
<td>20.00</td>
<td>4.69</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>LVP</td>
<td>73.54</td>
<td>7.08</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Rg</td>
<td>41.85</td>
<td>6.77</td>
<td>0.01</td>
<td>0.05</td>
<td>0.53</td>
</tr>
<tr>
<td>Ch</td>
<td>32.00</td>
<td>5.77</td>
<td>0.02</td>
<td>0.25</td>
<td>0.95</td>
</tr>
</tbody>
</table>

4.5 Discussion

4.5.1 Gene flow decline despite evidence of long-distance dispersal

Levels of contemporary gene flow between all pairs of subpopulations were low, which is consistent with earlier assumptions for sedentary behaviour of the Grey-crowned Babbler (King 1980). However, evidence for contemporary gene flow over relatively large geographic distances (e.g. $\geq 170$ km between Kerang south/Boort and Violet Town south) aligns with more recent studies that suggest the species capacity for dispersal may be higher than early estimations (Edwards 1993a; 1993b; Blackmore et al. 2011). Similarly, Johnson and Brown (1980) proposed that an absence of inbreeding in a population of banded Grey-crowned Babblers was due to birds dispersing beyond their local neighbourhood. Our results suggest the Grey-
The Grey-crowned Babbler is capable of dispersing large (>50 km) distances beyond their local genetic neighbourhood in our study system. The Chiltern to Rutherglen migratory route suggested slightly elevated contemporary gene flow rates (0.07) in comparison to most other gene flow rates between subpopulations. Groups of Grey-crowned Babblers between Chiltern and Rutherglen are separated by relatively small-moderate distances (range: 4-50 km). High levels of structural connectivity between these areas is provided by dispersed tree cover (Stevens et al. in press; Chapter 3), and roadside and riparian corridors (Fig. 4.1; pers. obs.). Birds are known to rely on corridors as conduits for dispersal and these types of habitat linkages can be crucial to animal movement in fragmented landscapes in agricultural systems (van der Ree and Bennett 2001; Gillies and St. Clair 2008; Vergara et al. 2013). Ongoing gene flow between between Chiltern and Rutherglen indicates bird movement between the two may be facilitated by both corridors and dispersed (stepping-stone) habitat connectivity.

Gene flow that is affected by habitat modification is evident in (sub)populations from low immigration rates (Harrisson et al. 2013). Both the east and west regions recorded major declines in the effective number of migrants per generation over time (94% and 98% respectively), showing immigration has declined markedly and is likely to increase the risk of regional extinction. In the west region, Kerang south/Boort had a relatively high number of private alleles (n = 7), suggesting recent isolation and genetic drift may have occurred. Although Kerang/south Boort recorded the highest contemporary gene flow rate (0.17; to Violet Town south) and which was over a large distance, the absence of these seven alleles in other sampled subpopulations may indicate that some birds emigrating from Kerang south/Boort
are not reproducing, are dying en route, or that the observed gene flow may be the genetic signatures from migrants arriving before the occurrence of the likely recent isolation. Although evidence from gene flow rates and first generation migrants supports long-distance emigration from Kerang south/Boort to Violet Town south, the level of gene flow has declined to levels that may not be sufficient to mitigate the long-term effects of immigration decline.

In the east region, Lurg/Violet Town north/Peechelba shows evidence of genetic bottlenecks over an extended period (i.e. significant results for both BOTTLENECK approaches). Low contemporary levels of gene flow between Lurg/Violet Town north/Peechelba and Violet Town south and Lurg/Violet Town north/Peechelba and Rutherglen, are surprising given long-term (>21 years) and large-scale (>1,200 ha) habitat restoration in the Lurg district that have led to substantial increases in Grey-crowned Babbler numbers (Thomas 2009; Vesk et al. 2015). Ongoing research into the effects of the restoration program on the Lurg (sub)population of the Grey-crowned Babbler have demonstrated a significant increase in population size over two survey time periods (2001-2008, mean = 59; 2009-2015, mean = 106) and an increase in the average group size (0.8 birds per group) (Thomas 2009; Vesk et al. 2015; Lacey, unpub. data; Moylan, unpub. data).

Although substantial areas of revegetated habitat is supporting major population increases within the Lurg district, this is a localised phenomenon amongst our study population. There remain large gaps of structural connectivity and a lack of habitat available between Lurg and subpopulations elsewhere, which may explain the low levels of gene flow between them.
4.5.2 Signatures of genetic bottlenecks and small effective population sizes

Significant bottleneck signatures are likely driven by declines in population size and/or reduced gene flow (Corneut and Luikart 1996; Broquet et al. 2010). Detectable signatures of bottlenecks generally become apparent when high levels of population decline have occurred or numbers of breeding individuals are reduced to unsustainable levels (i.e. $N_e<100$ individuals) (Peery et al. 2012). Strong evidence of longer-term signatures of bottlenecks in all subpopulations except for Kerang north, supports our estimates of small $N_e$ and evidence of drift. The Grey-crowned Babbler has undergone major population declines (Environment Australia 2000; 2011), and we suggest our results are related to recent isolation and/or population collapse as a consequence of habitat loss and fragmentation.

Small $N_e$ and severe reductions in $N_e$ can lead to a loss of fitness through inbreeding depression and reduced evolutionary potential (Frankham et al. 2014). For species of conservation concern, identifying populations which have small $N_e$ and that show evidence of recent bottlenecks is crucial for effective conservation decisions (McCusker et al. 2014). Mutation-scaled long term $\theta$ and contemporary $N_e$ showed similar patterns across both time periods of marginally higher estimates for the east region than the west, and were well below the suggested level to limit loss of fitness to ≤10% over five generations (Frankham et al. 2014). The census population in the southern extent of the species range is estimated at ≤2,000 individuals (Davidson and Robinson 2009). Samples used in this study were collected within the same census population and our results suggest that a low $N_e$ value is likely to hold true across the entire population, and which is below the number
required for the future genetic viability of these populations (i.e. >1,000) (Frankham et al. 2014).

4.5.3 Influences of drift and migration shaping contemporary population structure

Despite evidence for dispersal over large geographic distances, the number of migrants per generation dispersing between east and west regions in the study area is below the number required for mutation-drift equilibrium (Luikart et al. 1998). Our study supports a temporal decline in effective migration levels and is consistent with earlier studies indicating habitat fragmentation impacts on the dispersal of the Grey-crowned Babbler (Environment Australia 2011). This result is becoming commonplace amongst studies investigating the effects of habitat modification on population genetic structure and functional connectivity for species (Dutta et al. 2013; Harrisson et al. 2013; McCusker et al. 2013). Declines in genetic exchange between small populations particularly, are likely to be associated with increased levels of inbreeding and elevated risk of local extinction as subpopulations lose genetic diversity (Sunnucks 2011). Analyses indicated that Violet Town south was no longer providing gene flow to other subpopulations, which suggests a decrease in genetic exchange from this subpopulation. Long-term survey data in this area (Robinson, unpub. data) show population decline and extirpation of groups from habitat patches (Robinson 2006). The lack of emigration from Violet Town south, population decline and local extinctions is a concerning trend, adding to which the drift model estimated a 70% probability of drift to have occurred. Such evidence strongly indicates Violet Town south may experience increasing risks from negative effects of inbreeding and drift, and its long-term viability without intervention needs
to be questioned.

4.6 Conclusion and recommendations

Our results indicated that a substantial decrease in landscape-level structural connectivity in the study area has reduced the functional connectivity of the Grey-crowned Babbler. Conservation efforts such as species translocation and habitat connectivity enhancement requires information of functional connectivity and genetic variability of populations. The data we have presented is highly relevant to drive revegetation programs between subpopulations that have become disconnected, but also to inform a carefully managed translocation program (Weeks et al. 2011). We suggest that implementation of such interventions be urgently considered for a metapopulation that appears to be slipping out of equilibrium due to reduced functional connectivity (Volpe et al. 2014).

Arguments warning against translocation of species often suggest that mixing genes between previously genetically isolated populations can be detrimental to their long-term population genetic viability (Storfer 1999). This study provides strong indication that historically, these (sub)populations of Grey-crowned Babbler were mobile across the study area. Although the reported gene flow has declined to such low levels, nevertheless dispersal is detectable between regions suggesting that gene flow continues to occur. Such intervention programs could potentially be implemented as an interim measure until habitat revegetation can start to provide landscape connectivity in these areas. Increasing the landscape connectivity to facilitate gene flow for Grey-crowned Babblers will also provide long-term benefit for small woodland bird species that are affected by loss of habitat and invasive species in the same areas (Clarke and Oldham 2007).
Subpopulations in this fragmented landscape present a model for species that persist at the extremes of their range. But perhaps more importantly, here they also present a transferable model with broad applicability for many declining global avian species. This study has detailed how genetic approaches can be used to drive intervention-orientated conservation programs that aim to facilitate long-term gene flow in a contemporary landscape.
Modelling landscape-level structural and functional connectivity pathways for dispersal and gene flow of a declining cooperatively-breeding avian species.

A Grey-crowned Babbler group overcoming another anthropogenic barrier? (Actually, it was the ant-hill (bottom right) that caused the birds’ greater consternation!)
5.1 Abstract

Loss of structural connectivity can have major implications for the demography and gene flow of species in human-modified landscapes. The Grey-crowned Babbler (*Pomatostomus temporalis*) is a woodland bird severely affected by habitat loss and fragmentation in south-eastern Australia. We investigated the relationship between landscape structure and genetic distance for subpopulations of this species. This research is based on genetic data from 135 sampled individuals from geographically separated subpopulations. We implemented an original and reciprocal causal modelling framework and compared alternative hypotheses of factors influencing gene flow by correlating matrices of pairwise genetic distances with two measures of distances among individuals: matrices of Euclidean distance (i.e. isolation-by-distance (IBD)) and distances based on measures of landscape resistance (i.e. isolation-by-resistance (IBR)). Correlations were estimated with marginal Mantel and partial Mantel tests ($r$). Partial Mantel $r$ was used to test each candidate model (i.e. Euclidean and resistance distances) against all other alternative models. Marginal Mantel $r$ provided greater support for isolation-by-resistance over isolation-by-distance. The highest supported isolation-by-resistance model for males showed partial Mantel $r$ was higher than the marginal Mantel $r$, indicating isolation-by-resistance had a greater effect than isolation-by-distance on male movement at a landscape-level. Marginal Mantel tests indicated an isolation-by-resistance model as the best fit for genetic distance among female pairs which incorporated moderate resistance values for treeless areas, a resistance effect for major roads and a resistance effect for unsuitable vegetation at elevations >300m. A sex-specific
genetic response to landscape resistance was evident from significantly higher marginal Mantel \( r \) values for females than for males, indicating a stronger influence of landscape structure on female dispersal. Reciprocal causal modelling identified one isolation-by-resistance model incorporating the highest resistance values for treeless areas that best explained genetic distances of the Grey-crowed Babbler in the study area. Riparian and roadside habitat was identified as potential pathways across the landscape. Conservation of the Grey-crowned Babbler will therefore be enhanced by identifying areas of habitat with low landscape resistance for potential translocation sites, and gaps in structural connectivity for prioritising restoration works in areas that lack tree cover, to promote genetic exchange between disconnected subpopulations.

### 5.2 Introduction

#### 5.2.1 Conserving the genetic viability of populations in large fragmented landscapes

Population genetic structure is influenced by a number of ecological factors, including: natural and anthropogenic modification of habitat; the rate and spatial extent of habitat change; stochastic and deterministic processes; and species life history traits (e.g. dispersal ability) (Sunnucks 2011; Cushman et al. 2013a). Habitat loss and fragmentation can directly affect the amount of habitat available and the ability of animals to move through landscapes (Van Houtan et al. 2007; Pavlacky et al. 2012; Ortego et al. 2015). Severe or ongoing habitat fragmentation can isolate populations and expose them to threats associated with small population size, such as the loss of genetic variability arising from genetic drift or inbreeding processes.
(Kupfer et al. 2006). Loss of genetic diversity in fragmented systems reduces the evolutionary potential of populations and their resilience to withstand stochastic events, disease and climate change (Beckman et al. 2007; Amos et al. 2014; Nimmo et al. 2015).

Maintaining species’ genetic variability and persistence in landscapes that have undergone broad-scale habitat modification requires both local and long-distance dispersal (Kiester et al. 1982). Dispersal increases the likelihood of re/colonisation of vacant habitat and counteracts the negative effects of genetic drift (Amos et al. 2014); while immediately, and potentially in the future, increasing population size. Therefore, landscape features that facilitate the dispersal of a species, such as habitat stepping-stones or riparian corridors (Cushman et al. 2009; Christie and Knowles 2015), are critical elements of landscape connectivity for species vulnerable to fragmentation effects.

Landscape connectivity, refers to the degree to which landscape structure facilitates or impedes movements of organisms between resource patches, and is species-specific (Taylor et al. 1993; Cushman et al. 2009). Landscape connectivity has two aspects: structural connectivity and functional connectivity. Structural connectivity refers to a landscape’s physical elements and configuration, while functional connectivity refers to an organism’s ability to move through the landscape (Tischendorf and Fahrig 2000), both of which are important for population persistence in modified landscapes. Conservation plans which aim to promote dispersal and gene flow for vulnerable species should ensure the preservation of landscape connectivity at appropriate spatial scales to provide coherent pathways for movement (Sunnucks 2011; Ruiz-Gonzalez et al. 2014).
Landscape genetics is increasingly being used to understand connectivity in fragmented landscapes (Manel et al. 2003; Storfer et al. 2007; Cushman et al. 2013a). Landscape genetics combines landscape ecology, population genetics and spatial statistics. By comparing the spatial distribution of selectively neutral genetic markers (i.e. those most suited to estimate genetic history, gene flow and dispersal) with the spatial arrangement of environmental features across a landscape, inferences may be made about the influences and pattern of individuals’ movement and gene flow (Manel et al. 2003; Holderegger et al. 2006). Further developments in analytical methods such as spatial landscape models, causal modelling, and reciprocal causal modelling, frameworks (Cushman et al. 2006; McRae and Beier 2007: Cushman et al. 2013a) provide beneficial tools which can increase the computational capability, statistical power and validity of results required for complex analyses processes. Information of genetic variation patterns and the correlation with landscape features is particularly useful for conservation management of species experiencing landscape-scale habitat loss. Understanding landscape connectivity in declining populations is paramount to instruct effective short- and long-term conservation measures that aim to facilitate genetic variation and population viability of species at risk. Translocation of a species or habitat enhancement for example, require genetic information on landscape-scale population genetic connectivity and variability. It is critical to identify suitable habitat areas for trialling translocation or habitat that requires rehabilitation, to increase the potential for genetic exchange between existing and translocated populations (Shanahan and Possingham 2009).
5.2.2 Evidence of gene flow between declining species requiring genetic investigation

In Australia, land clearing for agricultural production, resource extraction, urbanisation, and development of infrastructure (e.g. roads) has resulted in broad-scale changes to native vegetation (Reid and Landsberg 1999; Bradshaw 2012). Widespread habitat loss and fragmentation have been closely linked with avian declines (Ford et al. 2001). In many regions, such processes have reduced functionally connected ecosystems to agricultural mosaics scattered with disparate and degraded habitat patches (Bradshaw 2012), and has led to population declines and extirpation of numerous species (Ford et al. 2009; Szabo et al. 2011). Anthropogenic landscape modification continues to affect the population viability and persistence of many species in Australia and across the globe (Environment Australia 2011; Ceballos et al. 2015).

The Grey-crowned Babbler (*Pomatostomus temporalis*) is a woodland bird species that has been severely affected by the loss and fragmentation of its habitat in south-eastern Australia (Robinson 2006; Blackmore et al. 2011; Stevens et al. in press; Chapter 3). The Grey-crowned Babbler lives in social groups consisting of a dominant breeding pair assisted by ‘helpers’ (usually previous offspring) (Brown and Brown 1981). Local population neighbourhoods are characterised by closely-related individuals (Koenig et al. 1992; Blackmore et al. 2011; Stevens et al. in press; Chapter 3). The range of the eastern subspecies’ *Pomatostomus temporalis temporalis* incorporates approximately two-thirds of eastern Australia (Fig. 5.1). Once common throughout much of this range, the species has undergone significant contraction in distribution and population decline (>90%) (Robinson 1993; Department of
Environment and Heritage 2013). This decline is largely recorded from areas across the southern extent of the subspecies distribution, and is attributed to the loss and fragmentation of temperate eucalypt woodlands (Environment Conservation Council 1997; Robinson 2006; Environment Australia 2011). Extant groups of the Grey-crowned Babbler are restricted mostly to roadside or riparian vegetation, small patches of adjacent remnant woodland within farmland (<0.5 ha) and the edges of the few remaining larger conservation reserves (>5 ha) (Robinson 2006).

The map of Australia includes the study area (black rectangle). The main map of the study area shows: regions (black rectangular outlines); sampling sites (black triangles) that are associated with subpopulations (labels); tree cover (vegetation cover >2 m in height) (grey shading).

Figure 5.1 Map of Australia indicating the location of the study area within Victoria, and site locations within the study area

Recent studies of gene flow conducted in a highly fragmented landscape in the
southern parts of the subspecies’ range at different spatial scales (Stevens et al. in press; Chapters 3 and 4) contrasts with previous understanding of its movement ecology (King 1980; Robinson 1993b). Stevens et al. (in press; Chapter 3) suggested that strong patterns of isolation-by-distance amongst females indicated that females were more philopatric than males. In some cases, gene flow had occurred across large geographical distances (~220 km), with a male-biased trend in long-distance dispersal records (Stevens et al. in press; Chapters 3 and 4). Furthermore, methods used to estimate levels of historical gene flow (i.e. MIGRATE, (Beerli and Felsenstein 2001; Beerli and Palczewski 2010)) showed contemporary levels of gene flow to have undergone major decline (up to 98%) relative to historical gene flow (Chapter 4).

Understanding functional connectivity of habitat (i.e. how land cover and other environmental attributes influence movement and gene flow) amongst spatially structured and declining populations is paramount for effective long-term conservation, to ensure the maintenance of genetic variation and population viability (Amos et al. 2014). There is a knowledge gap concerning landscape-scale dispersal of Grey-crowned Babbler populations in the southern part of the species’ distribution (Stevens et al. in press; Chapter 3). It is not known whether reported declines in population size and gene flow in this area can be explained by reduced movement and gene flow through treeless areas (i.e. isolation-by-resistance effects). This study builds on long-term (~30-yr) survey data and research on habitat requirements of the Grey-crowned Babbler in central and north-eastern Victoria (Robinson 1993; Tzaros 1995; Tzaros 2001; Davidson 2009; Lacey unpub. data; Robinson unpub.data). It investigates the relationship between landscape structural connectivity and genetic estimates of functional connectivity (i.e. individual mobility
and gene flow) between geographically isolated subpopulations of the Grey-crowned Babbler.

We hypothesised that dispersal movements of individuals would be enhanced by corridors of remnant vegetation and that large tracts of treeless farmland would inhibit dispersal and increase the genetic distance between Grey-crowned Babbler individuals. We also hypothesised that areas of unsuitable vegetation and the presence of major roads would constrain gene flow and be more pronounced in treeless areas. We predict that reduced genetic connectivity as a result of habitat loss and fragmentation has contributed to demographic decline in the southern part of the Grey-crowned Babbler’s range, and will manifest in landscape-level, isolation-by-resistance effects, whilst accounting for isolation-by-distance. Furthermore, we hypothesised that isolation-by-resistance would have a greater effect on females, the more philopatric sex, than males.

Specifically, this study addressed these hypotheses by:

1. formulating several spatially-explicit landscape-level models, including isolation-by-distance and isolation-by-resistance, for the Grey-crowned Babbler;

2. using a reciprocal causal modelling framework to test for isolation-by-resistance patterns amongst individual genetic distances whilst accounting for any isolation-by-distance;

3. investigating whether a genetic response was more pronounced in the more philopatric sex (females), and;

4. interpreting these results to provide information on important dispersal pathways for the conservation management of the Grey-
5.3 Methods

5.3.1 Sampling

Individual birds were sampled for blood at 40 sites selected from three geographic regions across northern Victoria: west (n = 15, Kerang; Boort), south-east (n = 12, Violet Town; Lurg) and north-east (n = 13, Peenelba; Rutherglen; Chiltern) (Fig. 5.1). These regions include five of the eight largest known populations of the Grey-crowned Babbler in Victoria (Davidson and Robinson 2009). Call playback was used to confirm the presence and size of a family group at each study site. An on-ground search using call playback was conducted in areas of habitat within a 2 km radius of each study territory to determine distances to adjacent groups. Group territories were verified from nesting activity and site locations were recorded on a Geographic Positioning System (GPS). Sampling took place from June 2010-April 2012, which incorporated two annual breeding seasons of the species. Birds were lured with call playback and trapped using mist-nets. Each individual was banded with a metal leg band provided by the Australian Bird and Bat Banding Scheme (ABBBS) and a unique combination of three coloured plastic leg bands for identification in the field. Individuals were weighed and measured, and a blood sample (~70 μl) collected from the brachial vein using a VITREX® capillary tube. Blood was transferred to a Whatman FTA Card® and stored at room temperature in paper envelopes. A total of 135 individual Grey-crowned Babblers were sampled across 40 sites, each site representing a discrete family group.

For discussion purposes, we assigned the sampled family groups to
subpopulations that we considered the most biologically meaningful (two subpopulations per study region, \( n = 6 \)) based on: a) earlier analyses that identified the genetic association of individuals (i.e. TESS (Chen et al. 2007)) (Stevens et al. in press; Chapter 3) and b) aligning groups according to location. The six subpopulations were arranged as: 1. Kerang north; 2. Kerang south and Boort; 3. Violet Town south; 4. Lurg, Violet Town north and Peechelba; 5. Rutherglen, and; 6. Chiltern (Fig. 5.1).

### 5.3.2 Molecular methods

Genomic DNA was isolated from a 2 mm\(^2\) blood-soaked sample taken from stored cards, using the QIAGEN DNeasy Blood and Tissue kit (QIAGEN Inc., Valencia, CA, USA) as per the protocol of the manufacturer. DNA isolates were quantified using a Qubit® fluorometer kit (Invitrogen). Sex was determined by amplifying the CHD-1 (chromo-helicase-DNA-binding) gene in 20 \( \mu \)l reactions containing 8 \( \mu \)l of Go Taq (Promega), 1 \( \mu \)l of avian sexing markers P2 and P8 (Griffiths et al. 1998) (10 \( \mu \)m) and 2 \( \mu \)l of template. PCR cycles consisted of: 94 °C for 3 min; then 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final elongation at 72 °C for 4 min. Sexes were identified from PCR products visualised by gel electrophoresis on E-Gel® SYBR® Safe 2% agarose gels (Invitrogen). DNA isolates were genotyped for 13 Grey-crowned Babbler microsatellite loci: Pte101, Pte102, Pte103, Pte105, Pte106, Pte108, Pte109 (Kawano et al. 2007), Pte17, Pte24, Pte42, Pte47, Pte48, Pte50 (Blackmore et al. 2006). The forward primer of each locus was labelled with an appropriate fluorescent tag: FAM (GeneWorks), NED, PET or VIC (Applied Biosystems). Samples were subsequently genotyped by the Australian Genomic Research Facility on an AB3730 capillary sequencer and analysed using Genemapper.
**5.3.3 Microsatellite performance and pairwise individual genetic distance**

Microsatellite performance of loci for all samples was assessed using Genepop v 4.2 (Raymond and Rousset 1995) to test for deviations from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium, implementing a Bonferroni correction for multiple comparisons (Rice 1989). Loci were checked for the presence of null alleles by looking for consistent departures from HWE in the direction of homozygous excess. Genotypic data for loci were manually checked for sex-linkage. GenAlEx v 6.5 (Peakall and Smouse 2006) was used to calculate the mean expected and observed heterozygosity of loci within each subpopulation.

To enable inferences about the effect of landscape on genetic structure of a continuously distributed species, we calculated pairwise individual genetic distances in GenAlEx v 6.5 (Peakall and Smouse 2006) for each pair of individuals, and for each sex separately. Genetic distance analyses resulted in three matrices which were subsequently used to compare against landscape distances as described in section 5.3.6 (see below).

**5.3.4 Building a landscape for resistance modelling**

Connectivity between points on the landscape surface can be estimated by assigning the relative difficulty of crossing a raster cell with a given value and calculating the aggregate difficulty of movement (‘resistance distance’) of an individual between the two points (Adriaensen et al. 2003). Resistance is the inverse to connectivity and can be used to estimate gene flow across landscapes. A number of methods are available to estimate landscape resistance (e.g. least-cost-paths;
Circuitscape) and are computed in a GIS platform (Adriaensen et al. 2003; McRae and Beier 2007). While the least-cost-paths method identifies a single optimal pathway by discounting all suboptimal routes, Circuitscape is based on electrical current theory and takes into account all possible paths between two points (McRae and Beier 2007).

To identify landscape features in the study area that may act as impediments or conduits for gene flow of the Grey-crowned Babbler, we formulated a geographic area for the spatial modelling. The modelling landscape was a minimum convex polygon which incorporated all sampling locations (n = 40) (Fig. 5.1) with an added 50 km buffer encircling the minimum polygon to mitigate effects arising from increasing resistance values at artificial boundaries (Koen et al. 2010). The area outside the boundary was assigned a ‘no value’ and excluded from analyses. Resistance layers were constructed using ARCGIS v 10.1 (ESRI 2010) and the output was formatted to ASCII grids using the Export to Circuitscape Tool (Jenness 2010). The raster cell size for all layers was scaled to 100 m, a resolution considered as a suitable compromise between the functional grain relevant for the study species (i.e. less than the known minimum group territory size of 2 ha), study area size (i.e. computational load) and detectability of landscape elements predicted to affect gene flow (e.g. patches and scattered tree cover; major roads) (Department of Sustainability and Environment 1990-1999; Amos et al. 2012; Ruiz-Gonzalez et al. 2014). GIS layers provided a suite of raster data sets (n = 13) representing different hypotheses (i.e. models) for the resistance of different landscape features on gene flow of the Grey-crowned Babbler.
5.3.5  

**Building landscape distance models**

5.3.5.1  **Null model surface**

A null model analogous to isolation-by-distance (IBD) was produced by using a raster in which all cells were allocated a uniform resistance value of 1 (Wright 1943; Amos *et al.* 2012) (Table 5.1). This model assumes a homogenous resistance to movement of individuals across the entire study area and was denoted as ‘isolation-by-distance’. The calculated values in this model were appropriate to later use in partial Mantel tests to factor out the effects of Euclidean distance from effects associated with landscape features.
<table>
<thead>
<tr>
<th>Model groups</th>
<th>Resistance surface/model code</th>
<th>Tree cover</th>
<th>No-tree cover</th>
<th>Elevation (&gt;300 m)</th>
<th>Major roads</th>
<th>Nearest neighbours</th>
<th>All land cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation-by-distance</td>
<td>UNIFORM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Binary landscapes</td>
<td>tr5</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>tr50</td>
<td>1</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>tr100</td>
<td>1</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneous landscapes</td>
<td>mosaic5</td>
<td>1</td>
<td>5</td>
<td>100</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mosaic50</td>
<td>1</td>
<td>50</td>
<td>100</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mosaic100</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binary landscapes including nearest neighbours</td>
<td>tr5NN</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tr50NN</td>
<td>1</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tr100NN</td>
<td>1</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Heterogeneous landscapes including nearest neighbours</td>
<td>mosaic5NN</td>
<td>1</td>
<td>5</td>
<td>100</td>
<td>75</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>mosaic50NN</td>
<td>1</td>
<td>50</td>
<td>100</td>
<td>75</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>mosaic100NN</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
### 5.3.5.2 Surfaces based on tree cover

A binary set of models \((n = 3)\) were formulated by assigning resistance values to landscape features that were either favourable or unfavourable for landscape movement of the species (i.e. tree cover versus no-tree cover). Cells identified with tree cover were given the lowest resistance value of 1 (Table 5.1). No general rules exist for the allocation of resistance values to non-habitat areas (Ruiz-Gonzalez et al. 2014). Therefore, we sought to determine the strength of resistance for no-tree cover and to confirm the detectability of resistance. We created models with different resistance values assigned to no-tree cover \((5, 50\text{ or }100)\) and which represented different alternative resistance surfaces to landscape movement (e.g. a resistance value of 5 for a no-tree cell is 5 times harder for a bird to move through than a treed cell with a value of 1). Models based on these surfaces were denoted ‘tr’ with a suffix representing the different values assigned to no-tree cover \((\text{tr5; tr50}; \text{tr100})\) (Table 5.1).

### 5.3.5.3 Surfaces based on landscape resistance heterogeneity

Two ecological constraints on landscape movement of the Grey-crowned Babbler were incorporated into six landscape resistance models. These models provided resistance surfaces informed by ecological attributes previously identified as relevant to the distribution of the Grey-crowned Babbler. First, habitat of the Grey-crowned Babbler occurs in areas of relatively low elevation (i.e. on grassy woodlands of the plains rather than forests on inland slopes of the ranges) (Department of Sustainability and Environment 2007; Department of the Environment 2015). Second, road infrastructure is known to influence the presence of some bird species (Develey and Stouffer 2001; Parris and Schneider 2009; Jack et
al. 2015) and Grey-crowned Babblers have been shown to avoid roads with high traffic volume (>2,000 vehicles per day) in parts of this study area (van der Ree et al. 2008). Non-habitat at higher elevations and proximity to major roads are likely to increase landscape resistance, even though tree cover may be present. Therefore, we formulated models that incorporated heterogeneous resistance levels to better reflect a realistic filtering effect of landscape resistance on the species (Sunnucks 2011). These models were based on the ‘tr’ models and included resistance values for elevation and major roads (Table 5.1). Areas with elevation >300 m within the modelling area were assigned the highest possible resistance value of 100, because the species does not inhabit forested areas >300 m elevation. Major roads with traffic volume of >2,000 vehicles per day were also included in models and assigned a value of 75. We assumed a major road would not pose a complete barrier to movement, especially where roadside vegetation and vegetation in the surrounding landscape was present. This resulted in the inclusion of three major roads: the Hume Highway; Murray Valley Highway; and Violet Town-Shepparton Road. Models based on these surfaces were denoted ‘mosaic’ and allocated the same suffix stipulating values for no-tree cover (mosaic5; mosaic50; mosaic100) (Table 5.1).

5.3.5.4 Surfaces based on nearest neighbours

The Grey-crowned Babbler is a cooperatively-breeding species with high site-fidelity, and local populations are generally comprised of family groups located within 1.5 km from another group (Robinson 1994). This study used 30 years of exhaustive census data on the Grey-crowned Babbler (Robinson 1993; Tzaros 1995; Tzaros 2001; Davidson 2009; Lacey unpub. data; Robinson unpub.data), a novel scenario for landscape-scale studies (Sunnucks 2011). To build further on the
landscape resistance models described above, six additional models were developed that included all known Grey-crowned Babbler groups mapped across the study area over the previous 30 years. We assigned the lowest resistance value to the location of each group (i.e. 1) (Table 5.1). These models were distinguished from other models by using the suffix ‘NN’ (tr5NN; tr50NN; tr100NN; mosaic5NN; mosaic50NN; mosaic100NN) (Table 5.1).

5.3.5.5 Formulating distance matrices and current maps using Circuitscape

The Euclidean and resistance distances between each pair of individuals, and each pair of males and females separately, were calculated with Circuitscape v 4.0 (McRae 2006) using the ‘connection between eight cells’ option. Circuitscape calculates the resistance distance between specified points of all possible paths using electrical circuit theory (McRae 2006). Pairwise resistance distances were calculated by using each cells maximum allocated value as its resistance level, thereby producing a surface resistance throughout each model. Circuitscape provided pairwise landscape distance matrices representative of an isolation-by-distance (IBD, i.e. Euclidean distance) and 12 isolation-by-resistance (IBR) models across the study area.

Circuitscape generates landscape ‘current’ maps based on electrical current flow between nodes (sampling sites) and represents the modelled resistance surfaces. High current levels are associated with areas of high connectivity (e.g. broad swathes of habitat). Current is typically highest around points of origin (i.e. sample location). These maps provide visual representation of conductance and resistance levels of landscape connectivity specified for a landscape model. Current
maps were produced for all models and used to identify important areas likely facilitating or constraining movement through the study area. Current maps can also assist with setting priorities for landscape connectivity restoration works for conservation management, or alternatively, identifying high connectivity areas for potential translocation sites between subpopulations.

5.3.6 Assessing relationships between genetic and landscape distances using a reciprocal causal modelling framework

Output from each of the three, pairwise genetic-distance matrices (i.e. all birds; males; females) were correlated with all landscape distance matrices, resulting in a total of 780 pairwise comparisons. Comparisons included a) the Euclidean distance, to investigate whether gene flow followed an isolation-by-distance pattern of movement (null model), and b) resistance distances calculated from each of the 12 landscape resistance models, to infer the effect of landscape features on long-distance gene flow of the study species. The correlations ($r$) between distance matrices were estimated with marginal Mantel (Mantel 1967) and partial Mantel (Smouse et al. 1986) tests as implemented in the package ‘ecodist’ in R v 3.1.2 (Goslee 2007; R Development Core Team 2006) with 10,000 permutations.

Mantel tests can be used to compare two matrices (Mantel 1967) and have very low Type II error rates (Cushman 2013). These methods are particularly reliable at identifying relationships between landscape resistance and genetic differentiation independent of null models based on isolation-by-distance (Amos et al. 2014). Earlier controversy of the reliability of Mantel tests (Balkenhol et al. 2009; Graves et al. 2013) can be partially allayed from recent studies that provide a number of ways to reduce the risk of Type I error associated with identifying spurious models of
Several effective approaches are now available, one of which implements a causal modelling framework using partial Mantel tests to increase the power to correctly identify landscape resistance processes (Amos et al. 2012; Cushman et al. 2013a; Ruiz-Gonzalez et al. 2014). Furthermore, a more recent method implements a reciprocal causal modelling framework which employs partial Mantel tests effectively, based on the relative support for each candidate model and which incorporates a reciprocal causal modelling step in the optimisation process (Cushman et al. 2013a).

To identify the best fit model from the suite of models available, we used multiple methods to include both Cushman et al.’s (2006) original and reciprocal (Cushman et al. 2013a) causal modelling frameworks. The original causal modelling involved three calculations: 1) marginal Mantel tests between genetic distances and each of the 12 landscape resistance (isolation-by-resistance) models, 2) partial Mantel tests between genetic distances and isolation-by-resistance, partialling out the effects of isolation-by-distance, and 3) partial Mantel tests between genetic distances and isolation-by-distance, partialling out isolation-by-resistance effects. To infer an isolation-by-resistance effect on our data, outcomes are expected to produce a significant result for 1) and 2) and a non-significant or negative result for 3) as described above. Reciprocal causal modelling used partial Mantel tests to test each landscape distance model (i.e. Euclidean and resistance distances) against all other landscape distance models (Cushman et al. 2013a). This approach involved calculating a reciprocal $r$ value from the difference between: a) the partial Mantel $r$ of each candidate model partialling out each alternative model, and b) the partial
Mantel $r$ of each alternative model partiaUing out the candidate model. Reciprocal causal modelling also assists to guard against identifying spurious correlations as the best fit model (Cushman et al. 2013a). A model supported as the best fit to genetic distance will show all reciprocal $r$ between the supported model and every other alternative model as positive, and the reciprocal $r$ between each alternative model and the supported model as negative. Thus our study implemented a robust approach by incorporating a cross-validation process of the results (Cushman et al. 2013a; Amos et al. 2014).

5.4 Results

5.4.1 Analyses of genetic data

Across all subpopulations, the mean observed and expected heterozygosities was 0.72 and 0.68, respectively. All loci were polymorphic, with the total number of alleles ranging between 2-31 across loci. There were no consistent patterns of linkage disequilibrium between any pairs of loci across all samples. Conformance tests showed loci deviations from Hardy-Weinberg equilibrium within Kerang south (Pte50; Pte102) and Kerang north (Pte47). These deviations likely reflect population-specific effects arising from local population substructure (i.e. Wahlund effect). No loci showed consistent departure from either linkage or Hardy-Weinberg equilibria across subpopulations and all loci were retained for analyses.
5.4.2 Marginal Mantel correlations between genetic and landscape distances

All marginal Mantel $r$ values were significantly higher for landscape distance models that were correlated with genetic distance for females than the genetic distance for males (two-tailed $t$-test: $t_{24} = -15.4, p < 0.0024$). This difference of genetic response between sexes indicates that landscape structure has a significantly stronger effect on the movement of Grey-crowned Babbler females than males in this study area.

5.4.2.1 Isolation-by-distance

Marginal Mantel $r$ provided support for the isolation-by-distance model for all birds and for males and females. Females recorded a higher $r$ value amongst the three data sets which indicates a strong influence of increasing distances inhibiting their movement through the landscape (all birds, $r = 0.145, p < 0.001$; males, $r = 0.082, p < 0.001$; females, $r = 0.334, p < 0.001$) (Table 5.2).
Table 5.2 Summary of causal modelling framework results for marginal and partial Mantel tests showing Mantel correlation ($r$) and $p$ values for Euclidean and resistance distances correlated with individual genetic distances of the Grey-crowned Babbler. These results are for all sampled birds, and for males and females, separately.

There are three Mantel tests comprising causal modelling: 1) marginal Mantel tests between the candidate model and genetic distance (G*L); 2) partial Mantel tests between the candidate model and genetic distance, partialling out Euclidean distance (i.e. isolation-by-distance) (G*L/Dis); 3) partial Mantel tests between the Euclidean (i.e. isolation-by-distance) and genetic distances, partialling out the candidate model (G*Dis/L). For a candidate model to be supported, tests 1) and 2) must be significant, while test 3) must be either negative or non-significant. The isolation-by-distance (null/isolation-by-distance) model (*) shows only values for marginal Mantel tests when correlated with genetic distance. Models that are supported in each criterion are in italics. CM indicates if the model is supported within the causal modelling framework (Y/N).

<table>
<thead>
<tr>
<th>Model/Resistance values</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All birds</td>
<td>Males</td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G*L</td>
<td>G*L/Dis</td>
<td>G*Dis/L</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Landscape isolation-by-distance (null model)</td>
<td>0.145</td>
<td>&lt;0.001</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>0.082</td>
<td>0.0024</td>
<td>n/a</td>
<td>n/a</td>
<td>0.334</td>
<td>&lt;0.001</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binary landscape</td>
<td>Tree vs. no-tree cover</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolation-by- resistance models</td>
<td>5</td>
<td>0.150</td>
<td>&lt;0.001</td>
<td>0.072</td>
<td>0.065</td>
<td>-0.060</td>
<td>0.890</td>
<td>N</td>
<td>0.093</td>
<td>0.001</td>
<td>0.090</td>
<td>0.107</td>
<td>-0.079</td>
<td>0.863</td>
<td>N</td>
<td>0.340</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.155</td>
<td>&lt;0.001</td>
<td>0.088</td>
<td>0.023</td>
<td>-0.068</td>
<td>0.935</td>
<td>Y</td>
<td>0.111</td>
<td>&lt;0.001</td>
<td>0.133</td>
<td>0.021</td>
<td>-0.111</td>
<td>0.951</td>
<td>Y</td>
<td>0.343</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.156</td>
<td>&lt;0.001</td>
<td>0.092</td>
<td>0.018</td>
<td>-0.070</td>
<td>0.942</td>
<td>Y</td>
<td>0.116</td>
<td>&lt;0.001</td>
<td>0.143</td>
<td>0.014</td>
<td>-0.118</td>
<td>0.967</td>
<td>Y</td>
<td>0.345</td>
</tr>
<tr>
<td>Heterogeneous landscape isolation</td>
<td>5</td>
<td>0.133</td>
<td>&lt;0.001</td>
<td>-0.110</td>
<td>0.992</td>
<td>0.124</td>
<td>0.003</td>
<td>N</td>
<td>0.063</td>
<td>0.037</td>
<td>-0.118</td>
<td>0.951</td>
<td>0.129</td>
<td>0.031</td>
<td>N</td>
<td>0.351</td>
</tr>
<tr>
<td>by-resistance models</td>
<td>50</td>
<td>0.152</td>
<td>&lt;0.001</td>
<td>0.100</td>
<td>&lt;0.001</td>
<td>-0.088</td>
<td>1.000</td>
<td>Y</td>
<td>0.107</td>
<td>&lt;0.001</td>
<td>0.221</td>
<td>&lt;0.001</td>
<td>-0.210</td>
<td>1.000</td>
<td>Y</td>
<td>0.376</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.155</td>
<td>&lt;0.001</td>
<td>0.121</td>
<td>&lt;0.001</td>
<td>-0.107</td>
<td>1.000</td>
<td>Y</td>
<td>0.117</td>
<td>&lt;0.001</td>
<td>0.244</td>
<td>&lt;0.001</td>
<td>-0.229</td>
<td>1.000</td>
<td>Y</td>
<td>0.379</td>
</tr>
<tr>
<td>Landscape isolation- Tree vs. no-tree cover + NN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>by-resistance</td>
<td>5</td>
<td>0.119</td>
<td>&lt;0.001</td>
<td>-0.068</td>
<td>0.922</td>
<td>0.106</td>
<td>0.018</td>
<td>N</td>
<td>0.050</td>
<td>0.125</td>
<td>-0.065</td>
<td>0.822</td>
<td>0.092</td>
<td>0.066</td>
<td>N</td>
<td>0.288</td>
</tr>
<tr>
<td>including nearest</td>
<td>50</td>
<td>0.108</td>
<td>&lt;0.001</td>
<td>-0.056</td>
<td>0.866</td>
<td>0.111</td>
<td>0.011</td>
<td>N</td>
<td>0.047</td>
<td>0.182</td>
<td>-0.038</td>
<td>0.703</td>
<td>0.077</td>
<td>0.085</td>
<td>N</td>
<td>0.255</td>
</tr>
<tr>
<td>neighbours (NN)</td>
<td>100</td>
<td>0.107</td>
<td>&lt;0.001</td>
<td>-0.053</td>
<td>0.862</td>
<td>0.111</td>
<td>0.011</td>
<td>N</td>
<td>0.049</td>
<td>0.178</td>
<td>-0.031</td>
<td>0.671</td>
<td>0.072</td>
<td>0.098</td>
<td>N</td>
<td>0.252</td>
</tr>
<tr>
<td>Mosaic + NN</td>
<td>5</td>
<td>0.107</td>
<td>&lt;0.001</td>
<td>-0.116</td>
<td>0.993</td>
<td>0.151</td>
<td>0.001</td>
<td>N</td>
<td>0.028</td>
<td>0.269</td>
<td>-0.117</td>
<td>0.956</td>
<td>0.140</td>
<td>0.008</td>
<td>N</td>
<td>0.300</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.104</td>
<td>&lt;0.001</td>
<td>-0.073</td>
<td>0.933</td>
<td>0.125</td>
<td>0.004</td>
<td>N</td>
<td>0.034</td>
<td>0.263</td>
<td>-0.064</td>
<td>0.822</td>
<td>0.098</td>
<td>0.038</td>
<td>N</td>
<td>0.268</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.104</td>
<td>&lt;0.001</td>
<td>-0.065</td>
<td>0.903</td>
<td>0.120</td>
<td>0.006</td>
<td>N</td>
<td>0.038</td>
<td>0.240</td>
<td>-0.051</td>
<td>0.759</td>
<td>0.088</td>
<td>0.056</td>
<td>N</td>
<td>0.263</td>
</tr>
</tbody>
</table>
5.4.2.2 Isolation-by-resistance based on binary landscapes

Marginal Mantel $r$ showed support for all three binary (treed versus treeless areas) isolation-by-resistance models for all birds, and for males and females separately (Table 5.2). When these models included nearest neighbours (i.e. ‘tr,NN’), marginal Mantel $r$ showed support for all three models (i.e. ‘tr5NN’, ‘tr50NN’ and ‘tr100NN’) for all birds and for females. The same three models did not support male genetic distances (Table 5.2).

5.4.2.3 Isolation-by-resistance based on landscape resistance heterogeneity

Marginal Mantel $r$ showed support for the three heterogeneous isolation-by-resistance models (i.e. ‘mosaic,’) for all birds, and for males and females separately. Based on Mantel $r$ values, the marginal Mantel $r$ provided the highest support for the three isolation-by-resistance mosaic models for explaining female genetic distance across all marginal and partial Mantel $r$ values and amongst all three genetic distance data sets. Models ‘mosaic100’ and ‘mosaic50’ were in the top four supported models for all birds and for males. When nearest neighbours were included in mosaic models, correlations between genetic distances and ‘mosaic,NN’ models were supported for all birds and for females, but were not supported for males (Table 5.2).

5.4.3 Partial mantel correlations between genetic distance and landscape distance

5.4.3.1 Isolation-by-distance effects

Partial Mantel $r$ supported isolation-by-distance for females when isolation-by-resistance models that included nearest neighbours were partialled out (Table 5.2). Similarly, for all birds, partial Mantel $r$ supported isolation-by-distance when
isolation-by-resistance models incorporating nearest neighbours were partialled out, and when the model ‘mosaic5’ was partialled out. Partial Mantel tests for males showed support for isolation-by-distance after partialling out three of the isolation-by-resistance models (‘mosaic5’; ‘mosaic5NN’; ‘mosaic50NN’) (Table 5.2).

### 5.4.3.2 Isolation-by-resistance based on binary landscapes

After accounting for the effects of isolation-by-distance, the three isolation-by-resistance ‘trx’ models were all supported from partial Mantel tests for all birds and for males and females separately. When the binary models included nearest neighbours and isolation-by-distance effects were accounted for, none of the models were supported for all birds, and for males and females.

### 5.4.3.3 Isolation-by-resistance based on heterogeneous resistance landscapes

When isolation-by-distance was partialled out of mosaic models, partial Mantel tests supported two out of the three isolation-by-resistance models (‘mosaic100’ and ‘mosaic50’) for all birds, and for both males and females. In addition, the model ‘mosaic5’ was also supported for females. Partial Mantel r did not support any mosaic isolation-by-resistance models which included nearest neighbours (i.e. ‘mosaicNN’) with isolation-by-distance partialled out, for all birds, and for males and females separately.

### 5.4.4 Original and reciprocal causal modelling

Using the original method of causal modelling (Cushman et al. 2006), we found support for four landscape resistance models for all birds and for males, and ‘mosaic100’ given the greatest support. Although the marginal Mantel r showed a slightly higher value for the model ‘tr100’ for all birds (Table 5.2), both partial Mantel
tests reported higher values for ‘mosaic100’, indicating that this latter model explained more of the genetic distance for all birds than ‘tr100’. The original causal modelling method supported the three mosaic models (i.e. ‘mosaicx’) for female genetic distance and identified ‘mosaic50’ as the best fit model (Table 5.2).

Reciprocal $r$ indicated support (i.e. all reciprocal $r$ values between the supported model and every other alternative model are positive, and the reciprocal $r$ between each alternative model and the supported model is negative) for one isolation-by-resistance model for females, but could not distinguish between two isolation-by-resistance models for all birds and for males, from the 12 candidate models (Table 5.3). We determined from the two supported models for all birds and for males that the single fully supported model (i.e. the model with the highest difference of reciprocal $r$ between the two supported models) was ‘tr100’ for all birds, while the model ‘mosaic100’ was supported for both male and female genetic distances.
Table 5.3 Reciprocal causal modelling $r$ values estimated from partial Mantel $r$ differences between supported isolation-by-resistance models.

Results are provided for supported models for all birds ($n = 2$), for males ($n = 2$) and for females ($n = 1$). Values of competing supported models for all birds and for males only are indicated in bold, and the model with the highest difference between supported models (i.e. fully supported) is indicated with an asterisk (*).

| Alternative model | All birds | | | Males | | | | Females |
|-------------------|-----------|------------------|-----------------|------------------|------------------|------------------|------------------|
|                   | mosaic100 | tr100 | mosaic100 | tr100 | mosaic100 | tr100 | mosaic100 |
| tr100             | 0.002     |       | 0.020*    |       |           |       |           |
| tr50              | 0.012     | 0.096 | 0.044     | 0.192 |           |       | 0.179    |
| tr5               | 0.059     | 0.095 | 0.155     | 0.173 |           |       | 0.226    |
| mosaic100         |           |       | 0.020*    |       |           |       |           |
| mosaic50          | 0.159     | 0.043 | 0.265     | 0.051 |           |       | 0.099    |
| mosaic5           | 0.169     | 0.165 | 0.245     | 0.224 |           |       | 0.169    |
| tr100NN           | 0.128     | 0.134 | 0.126     | 0.120 |           |       | 0.294    |
| tr50NN            | 0.129     | 0.135 | 0.131     | 0.124 |           |       | 0.292    |
| tr5NN             | 0.127     | 0.133 | 0.156     | 0.143 |           |       | 0.268    |
| mosaic100NN       | 0.136     | 0.142 | 0.140     | 0.132 |           |       | 0.283    |
| mosaic50NN        | 0.140     | 0.146 | 0.149     | 0.140 |           |       | 0.280    |
| mosaic5NN         | 0.162     | 0.165 | 0.191     | 0.173 |           |       | 0.248    |
5.4.5  **Circuitscape landscape current mapping**

Current maps generated for all models provided useful tools to visualise differences between connectivity patterns of different landscape surfaces and to identify landscape connectivity for the Grey-crowned Babbler from current flows. The maps included here represent the most visually stark differences among the current maps available at the time of printing, to demonstrate the effectiveness for detailing differences between current flows (i.e. landscape distance models). The current map (Fig. 5.2) based on isolation-by-distance shows the highest current flows (yellow) to emanate from ‘nodes’ (i.e. sampling locations) and disperse in a graduated decline as landscape distance increases away from the nodes. A current map generated from the model ‘mosaic100NN’ indicated low landscape connectivity (Fig. 5.3). The inclusion of nearest neighbours in the map shows that even moderate connectivity between groups (green shading) disrupt gene flow, as these areas align with genetic breaks in the study population (Stevens *et al.* in press; Chapter 3).

Current maps that were generated from the two mosaic isolation-by-resistance models (‘mosaic50’, ‘mosaic5’; Figs. 5.4 and 5.5 respectively) show greater discernment between connectivity (i.e. current) levels. The highest landscape connectivity outside of the nodes, are aligned with riparian corridors (e.g. the Murray and Goulburn Rivers) and to a lesser extent, the smaller linear strips of roadside habitat, that are depicted most prominently in the east region (i.e. Chiltern, Rutherglen, Lurg/Violet Town north/Peechelba and Violet Town south). These current maps depicting landscape connectivity for landscape distance models, demonstrate the effect of elevation and major roads on gene flow of the Grey-crowned Babbler most evident in the map based on the ‘mosaic5’ model (Fig. 5.5)
130

Currents are drawn from the isolation-by-distance (null) resistance model, and represent isolation-by-distance using Euclidean distances. Colour shades indicate landscape current flows from areas of highest connectivity (yellow) to lowest connectivity (dark blue) (see colour ramp). Bright circles are sampling sites ($n = 40$) used as focal nodes and are representative of current origins. Subpopulations are indicated in black rectangles. All resistance values were assigned 1. Subpopulations: Chiltern (Ch); Rutherglen (Rg); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Kerang south/Boort (KSB); Kerang north (Kn).

Fig. 5.2 Current map based on isolation-by-distance and used to predict important connectivity areas between Grey-crowned Babbler subpopulations in southern areas of their range.
Fig. 5.3 Current map based on resistance values of the ‘mosaic100NN’ resistance model and used to predict important connectivity areas between Grey-crowned Babbler subpopulations in southern areas of their range.

Currents are drawn from the ‘tr100NN’ resistance model, and represent isolation-by-resistance using resistance distances. Colour shades indicate landscape current flows from areas of highest connectivity (yellow) to lowest connectivity (dark blue) (see colour ramp). Bright circles are sampling sites (n = 40) used as focal nodes and represent current origins. Subpopulations are indicated in black rectangles. Resistance values were assigned as: tree cover (1); no-tree cover (100); nearest neighbours (1; depicted as small circles scattered across the landscape). Examples of nearest neighbour representation (dots) are enclosed within the two red circles. Subpopulations: Chiltern (Ch); Rutherglen (Rg); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Kerang south/Boort (KSB); Kerang north (Kn).
Fig. 5.4 Current map based on resistance values of the ‘mosaic50’ resistance model and used to predict important connectivity areas between Grey-crowned Babbler subpopulations in southern areas of their range.

Currents are drawn from the ‘mosaic50’ resistance model, and represent isolation-by-resistance using resistance distances. Colour shades indicate landscape current flows from areas of highest connectivity (yellow) to lowest connectivity (dark blue) (see colour ramp above). Bright circles are sampling sites ($n = 40$) used as focal nodes and are representative of current origins. Subpopulations are indicated in black rectangles. Resistance values were assigned as: tree cover (1); no-tree cover (50); elevation >300 m (100); major road (75). Subpopulations: Chiltern (Ch); Rutherglen (Rg); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Kerang south/Boort (KSB); Kerang north (Kn).
Currents are drawn from the 'mosaic5' resistance model, and represent isolation-by-resistance using resistance distances. Colour shades indicate landscape current flows from areas of highest connectivity (yellow) to lowest connectivity (dark blue) (see colour ramp). Bright circles are sampling sites ($n = 40$) used as focal nodes and represent current origins. Subpopulations are indicated in black rectangles. Resistance values were assigned as: tree cover (1); no-tree cover (5); elevation >300 m (100); major road (75). Subpopulations are: Chiltern (Ch); Rutherglen (Rg); Violet Town south (Vs); Lurg/Violet Town north/Peecelba (LVP); Kerang south/Boort (KSB); Kerang north (Kn).

Fig. 5.5 Current map based on resistance values of the ‘mosaic5’ resistance model and used to predict important connectivity areas between Grey-crowned Babbler subpopulations in southern areas of their range.
5.5 Discussion

We employed a reciprocal causal modelling framework to compare alternative hypotheses of factors influencing gene flow (i.e. isolation-by-distance and isolation-by-resistance) between geographically separated subpopulations of the Grey-crowned Babbler. Landscape modification that produces a loss of structural connectivity poses an effective impediment for functional connectivity (e.g. dispersal and gene flow) of the Grey-crowned Babbler in the study area. Supported models demonstrated that isolation-by-resistance had the greatest influence on dispersal of the species and that landscape resistance amongst males and females separately, is most pronounced from a lack of landscape tree cover coupled with ecological and/or anthropogenic barriers.

Reciprocal causal modelling for both males and females supported one isolation-by-resistance model out of a possible 12. This model, ‘mosaic100’, indicated that gene flow of both sexes was facilitated by areas of land with tree cover and was constrained by a factor of 100 in areas without tree cover. Additionally, this single supporting model indicated that barriers which include areas of unsuitable vegetation (e.g. areas of elevation >300 m) and/or major roads, present greater resistance to gene flow of males and females than areas of tree cover. Including barriers in models improved correlations between genetic distances and isolation-by-resistance for males and females (Table 5.2). Results from original and reciprocal causal modelling suggest that population connectivity in the study area may be more vulnerable to habitat loss and
fragmentation processes that include barriers, as has been shown for other woodland/forest-dependant birds (Develey and Stouffer 2001; Bush et al. 2011; Cerame et al. 2014). Potential barrier effects from major roads and/or unsuitable vegetation areas may have a synergistic effect within fragmented landscapes. Barriers may reduce gene flow as a consequence of birds avoiding major roads lacking tree cover and areas of unsuitable vegetation (Forman and Alexander 1998; Ruiz-Gonzalez et al. 2014).

Comparable with other recent studies of landscape genetics, our results clearly indicate that using isolation-by-resistance models improves the ability to predict gene flow of a species in preference to relying only on an isolation-by-distance model (e.g. Roe Deer (Capreolus capreolus), Coulon et al. 2006; European Pine Marten (Martes martes), Ruiz-Gonzalez et al. 2014). Incorporating a number of approaches such as original and reciprocal causal modelling enabled us to refine our analysis process, thereby enabling the identification of a single best fit model for each of the three genetic distance data sets (i.e. all birds; males; females). Reciprocal causal modelling improved discrimination between alternate models and resolved Type I errors, by supporting only one model versus the original causal modelling support for 40% of all possible models.

5.5.1 Influences of structural connectivity on functional connectivity: insights into movement ecology of the Grey-crowned Babbler

Landscape genetic studies similar to this research have described different effects from variation in landscape connectivity for other avian species that are also vulnerable to habitat loss and fragmentation. Effects of landscape resistance on a suite of avian
woodland species for example showed that sedentary species were more affected than species that were more mobile or tolerant to fragmentation (Amos et al. 2014).

Landscape-level gene flow for the Grey-crowned Babbler is most likely restricted by areas lacking tree cover. Avoidance of areas with no tree cover by the species may lead to disrupted dispersal and vacant habitat patches left uncolonised (Harrisson et al. 2013).

This study provides new insights, substantiating earlier studies of the movement ecology of the Grey-crowned Babbler (Johnson and Brown 1980; King 1980) and complements recent knowledge of their movement at a fine-scale (<10 km) (Stevens et al. in press; Chapter 3). Stevens et al. (in press; Chapter 3) suggested that genetic response to landscape effects at a fine-scale was sex-specific as evidenced from strong isolation-by-distance patterns amongst females and which was not observed for males. Similarly, our results suggest a likely sex-specific response to landscape connectivity over larger spatial scales. We therefore suggest that in this study area, females are affected by different processes at different spatial scales (i.e. isolation-by-distance at fine-scale; isolation-by-resistance at landscape-scale) while males are constrained by landscape resistance.

Unexpectedly, models that included neighbouring groups had little influence on genetic distances of individuals within the study area. This species is known for its complex social behaviour and mating system and groups are located on average within 1-1.5 km of another group (Brown et al. 1983; Blackmore 2006; Stevens et al. 2015; Chapter 2). A lack of influence from neighbouring groups on the movement of
individuals in this study may indicate that previously recorded groups have suffered extinction, as some of the data used in landscape modelling relating to groups, dates back 30 years.

Road infrastructure and management (e.g. major highways) are suggested to affect Grey-crowned Babbler distribution (van der Ree et al. 2008) and show a higher association with roads that have moderate to low traffic volume (≤1,000 vehicles per day) rather than higher traffic volume (>2,000 vehicles per day). Our findings suggest that roads per se may not exert a strong influence on the gene flow of Grey-crowned Babbler, but more likely work in concert with a lack of tree cover such that a loss of structural connectivity along roadsides impacts the species presence and dispersal.

Previous research (Stevens et al. in press; Chapter 3) described gene flow of the Grey-crowned Babbler to be restricted between subpopulations separated by landscapes of ~5 km radius devoid of tree cover, and which included a major highway (i.e. Kerang north and Kerang south) (Fig 5.1). In contrast, pairs of subpopulations in the eastern region (Violet Town south and Violet Town north; Lurg and Violet Town north) were separated by similar distances but showed evidence of gene flow between subpopulation pairs. The gene flow in the east may result from higher roadside vegetation and connected habitat levels than is apparent in the west.

Our results from heterogeneous isolation-by-resistance models indicate that a lack of tree cover coupled with barrier effects from roads and/or unsuitable vegetation at higher elevation had negative effects on the species gene flow. However, allocating different resistance values to those used in this study, may achieve different results.
The degree of contrast in resistance to gene flow in habitat compared to non-habitat areas can affect whether a landscape configuration will result in significant effects (Rayfield et al. 2010; Spear et al. 2010). However, by testing different model configurations and including different data sets in our analyses, assisted to refine and validate our results.

We suggest that existing riparian corridors and networks of roadside vegetation are likely to be important components of structural connectivity that facilitate functional connectivity for this species in the study area. Evidence for long-distance (~170 km) gene flow between Kerang south and Violet Town south in a west to east direction (Chapter 4) is likely to be facilitated by high levels of connected tree cover. The production of current maps depict strong currents of landscape connectivity aligned with riparian corridors along the Murray and Goulburn Rivers (Figs. 5.3, 5.4 and 5.5). The Murray and Goulburn River systems may be providing important structural connectivity that facilitate landscape-level functional connectivity for the Grey-crowned Babbler. Gene flow amongst most eastern subpopulations (Rutherglen, Chiltern and Lurg/Violet Town north/Peechelba) is likely assisted by connected remnant roadside vegetation and higher levels of dispersed tree cover in the landscape, in addition to riparian corridors along small tributaries of the Murray River (Figs. 5.1, 5.3 and 5.4).
5.6 Management implications

Ecological networks are used in conservation management to maintain core areas of habitat and connections amongst them, with the aim of assisting species dispersal (Cushman et al. 2013b). These networks are identified and managed as a result of conservation planning processes which typically use expert opinion in the absence of empirical data (Ruiz-Gonzalez et al. 2014). Our investigations are critical in providing empirical data that has identified the effects of landscape resistance for a species of bird that has experienced recent major population declines. We suggest conservation plans for the Grey-crowned Babbler need to consider the mobility of both sexes at different spatial scales and incorporate opportunities to protect and enhance landscape connectivity. Landscape elements that we have identified as barriers (i.e. no-tree cover; unsuitable vegetation; major roads) and as potential conduits for gene flow (e.g. linear strips of tree cover) can be manipulated by land management to facilitate a greater level of genetic exchange between subpopulations. Our findings therefore may also prove relevant for investing in ecological network planning for similar fragmented landscapes as our study.

Identifying landscape areas with low resistance using current maps produced by Circuitscape may aid conservation management that aims to facilitate dispersal between unlinked subpopulations. Potential short-term conservation effort could consider translocation of Grey-crowned Babbler groups to vacant habitat areas, and thereby actively introduce new subpopulations with the potential to increase the overall
population size. Long-term conservation efforts should include identifying gaps in landscape connectivity and prioritising restoration works in these areas to create habitats through which Grey-crowned Babblers can disperse. Implementing conservation efforts through on-ground works such as revegetation and rehabilitation (e.g. corridor tree planting and remnant habitat enhancement) to connect subpopulations, especially smaller and more isolated subpopulations, will enhance functional connectivity for the Grey-crowned Babbler. Developing effective ecological networks will assist in the longer term persistence of this species, and will also assist other vulnerable species in the study area.
6 Conclusion and recommendations

A Grey-crowned Babbler breeding male (looking none too happy having had a blood sample taken or perhaps showing his concern about the species current status in their southern range extent??)
6.1 Thesis overview

This thesis focused on the effects of habitat loss and fragmentation on habitat quality for Grey-crowned Babblers and on their population processes. The research was prompted by the reported demise of local populations and declines in regional population sizes of the Grey-crowned Babbler in recent decades (Robinson 1994; Environment Australia 2011). To achieve this, I established a field sampling regime across much of the species’ southern range extant in the state of Victoria and carried out data analyses to assess indicators of habitat quality, population genetic structure, dispersal patterns, gene flow, and landscape connectivity. I compared findings from this study with relevant conclusions from 30 years of survey data and earlier studies of their habitat requirements undertaken in the southern parts of the species’ distribution, as well as with contemporary genetic research investigating their breeding ecology within northern areas of the species’ range.

6.1.2 Summary of key findings

This study provides new insight and a better understanding of the effects of habitat loss and fragmentation on a cooperatively-breeding, woodland-dependent species (Table 6.1). The Grey-crowned Babbler has suffered major population decline and range contraction, particularly in the southern part of its distribution in Australia, as a result of habitat loss and fragmentation (Robinson 2006; Environment Australia 2011). In addition, several threats to the species’ viability and long-term persistence in the
southernmost part of their range have been identified in this thesis, namely; i) signatures of bottlenecks and small effective population sizes; ii) disrupted dispersal between subpopulations; iii) major temporal declines in gene flow levels and; iv) adverse effects on movement ability through the landscape. The findings presented in this thesis assist to inform conservation actions that will benefit the Grey-crowned Babbler. The findings also serve as a case study of relevance to other sedentary and cooperatively-breeding species (e.g. Brown Treecreeper, Superb Fairy-wren), as well as other woodland-dependent species occupying fragmented ecosystems (e.g. Red-tailed Black Cockatoo) (Chapter 1).

6.1.1 Ecological investigations

Investigation of habitat quality for the Grey-crowned Babbler across parts of northern Victoria examined a range of ecological variables measured at different spatial scales (i.e. spatial stratification). These were assessed in relation to sites where family groups of the species were known to occur (Stevens et al. 2015; Chapter 2). I focused on demographic parameters (fledgling presence, family group size) as surrogates for territory quality (Blackmore and Heinsohn 2007).

Over one breeding season (June 2010 to April 2011), 58% of the study groups were observed with fledglings. Fledgling presence was strongly associated with group size, as previously reported for this species (Brown et al. 1983; Blackmore and Heinsohn 2007). Many cooperatively-breeding species have been shown to improve their breeding success with increasing group size (e.g. Florida Scrub Jay (Aphelocoma coerulescens), Mumme 1992; Daffodil Cichlid (Neolamprologus pulcher), Balshine 2001;
Meerkat (*Suricata suricatta*), Clutton-Brock 2001). However, no association was evident between presence and absence of fledglings and geographic region, or between fledgling presence and habitat variables measured at each location (Table 6.1).

The average group size was larger in the west than in the south-east and north-east regions of the study area. Habitat attributes within group territories also differed between the west and north-east, and the west and south-east regions, but no differences were evident between the north- or south-east regions. However, vegetation attributes that differed most between territories in different regions (e.g. litter cover) were not useful predictors of group size. Consequently, after controlling for region, no site- or landscape-scale variables were found to influence group size (Table 6.1). Agricultural land-use in the west is largely given to cropping, while the two eastern regions support both cropping and grazing. Fine-scale variation in landscape structure associated with different land-uses, such as differential retention of paddock trees, may contribute to regional differences in group size of the Grey-crowned Babbler (Gibbons and Boak 2002; Maron 2005).

Applying a multi-scaled spatial investigation of potential indicators of habitat quality was an endeavour to untangle ecological influences on demographic processes in this declining species. The lack of identified indicators of habitat quality, together with the known historical population decline of the species in this highly fragmented landscape suggests that the species is likely occupying the most optimal habitat left available, now largely found in roadside vegetation. Of conservation concern, roadside management (e.g. undergrowth removal), roadside development (e.g. road widening)
and stochastic events such as fire, all have the potential to adversely affect these small and linear remnants of native vegetation.
Table 6.1 Overview of thesis themes and broad findings for the ecology and biology of the Grey-crowned Babbler

*Pomatostomus temporalis*.

<table>
<thead>
<tr>
<th>Theoretical basis and focus of chapters</th>
<th>Objectives</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landscape ecology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chapter 2</td>
<td>Identify relationships between group size, habitat attributes and landscape parameters, to determine factors that influence territory quality and species persistence.</td>
<td>Identify relationships between fledgling presence, group size, habitat attributes and landscape parameters, to determine territory quality for breeding success.</td>
</tr>
<tr>
<td>Determinants of territory quality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular ecology</td>
<td>Estimate baseline genetic diversity metrics of the study population. Estimate relatedness between groups</td>
<td>Observed heterozygosity amongst all subpopulations was high. Higher numbers of private alleles were evident in the west than in the east regions. No differences among subpopulations were evident for other genetic diversity</td>
</tr>
</tbody>
</table>
Baseline genetic metrics, local group demography and dispersal patterns across the study population and individuals. Investigate individuals, and sex-bias, dispersal patterns. Establish population genetic structure of individuals across the study area. metrics used. Within-group relatedness was high at zero distance (within-group level), and described an isolation-by-distance relatedness pattern amongst all individuals. Females were affected up to 8 km from isolation-by-distance; males were not affected by isolation-by-distance beyond the group-level (>500 m).

A genetic signal for male sex-biased dispersal existed across the study area.

Four phylogenetic clusters were identified from DNA sequences of 135 Grey-crowned Babbler individuals that approximated the geographic sampling locations.

Gene flow among groups in the eastern region was evident from high levels of admixture among individuals.

| Landscape genetics Chapter 4 | Landscape-level gene flow and subpopulation connectivity | Investigate the impact that recent population declines may have on population connectivity and gene flow at the landscape level. Compare historical and contemporary gene flow rates per generation Estimate effective population size Check for signatures of bottlenecks | Low to very low rates of contemporary gene flow per generation were observed amongst all subpopulations and only two subpopulations displayed similar symmetric (low) gene flow rates between them. Historical gene flow levels per generation for a subset of emigration routes were more moderate and were significantly higher than contemporary levels per generation. Contemporary effective population size estimates were small and indicated numbers well below numbers that are |
among subpopulations suggested (i.e. >100 individuals) are required to limit a loss of fitness to ≤10% over five generations.

Alternative models for demographic history indicated all subpopulations to have experienced drift rather than being in migration-drift equilibrium, and bottleneck signatures were evident for five of the six subpopulations.

| Landscape genetics and conservation management | Mantel $r$ supported isolation-by-distance (IBD) and isolation-by-resistance (IBR) models but provided higher support for IBR. |
| Chapter 5 | Two IBR models with the highest resistance values for no-tree cover were identified as the best fit to data for all birds and for males separately, indicating IBR had a greater effect than IBD on movement at a landscape-level. |
| Landscape connectivity; contributions of landscape structural connectivity to Grey-crowned Babbler functional connectivity. | One IBR model incorporating the highest heterogeneous resistance values for no-tree cover, major roads and elevation levels representing landscape resistance and barriers was the best fit for females. Marginal Mantel $r$ values were higher for females than for either males or all birds, indicating a greater, and more pronounced, effect from landscape resistance for females. |
| | Current maps, representing connectivity through the landscape, were used to identify areas contributing most to connectivity between focal points (sampling sites). Maps depicted riparian corridors and networks of roadside vegetation as likely pathways for movement of the Grey-crowned Babbler in these landscapes. |
6.1.2 Biological investigations

Genetic analyses revealed much about the effects of habitat loss and fragmentation on the Grey-crowned Babbler (Chapters 3-5). These investigations revealed population genetic substructure, differences in genetic diversity (private alleles, i.e. alleles unique to a subpopulation) among regions, male-biased dispersal and patterns of isolation-by-distance influencing local movement of females (Chapter 3). Genetic drift and signatures of bottlenecks were evident in most subpopulations and levels of gene flow had suffered major temporal declines between east and west regions (Chapter 4). Both sexes were adversely influenced by landscape resistance and barriers to movement, which was more pronounced for females (Chapter 5). From these findings, I inferred that the population substructure and differences in genetic diversity result from a disruption to metapopulation processes, as well as a temporal decline in gene flow. Loss of habitat connectivity across the study area has resulted in isolation-by-resistance and, with the exception of one subpopulation, has led to bottlenecks amongst the other five subpopulations. The patterns of isolation-by-resistance and isolation-by-distance observed in the study area are most likely mediating declines in gene flow. It seems that the reported demographic population declines may have led to population genetic declines in the Grey-crowned Babbler in these parts of their southern range.
6.2 Conservation implications of fragmented landscapes

Habitat loss and fragmentation continue to impact global biodiversity, and published research continues to describe population declines, extinctions and disrupted ecological and biological processes for many species (Vitousek et al. 1997; Pimm et al. 2006; Essl et al. 2015). Mechanical agents of habitat modification such as fire, flood and agricultural practices continually alter landscape connectivity (Brown 2011). Threatening processes resulting from human-modification of habitats, such as disrupted dispersal patterns and decline in genetic diversity, can have major consequences for metapopulations in modified landscapes (Bouzat et al. 1998a; Drechsler et al. 2003; Elkin and Possingham 2008).

Whether initiated naturally or anthropogenically, changes in habitat availability and connectedness (e.g. total extent, patch size, spatial pattern of habitats) influence population processes considerably (Sunnucks 2011). Agricultural systems in particular, are major drivers of land-use change and conversion for these purposes typically results in extensive modification of ecosystems and their natural processes (Bennett et al. 2006). Such extensive changes to landscapes can greatly influence the distribution and abundance of individual species and lead to population fissures and declines in the genetic diversity of populations. For example, the trajectory of habitat loss resulting from agricultural intensification in North American grasslands has been shown to better explain the declines in grassland bird species than the decreasing use of toxic insecticides (Hill et al. 2014). Conversely, habitat loss and fragmentation can be of
benefit for some species such as disturbance specialists or new invaders that benefit from anthropogenic modifications (e.g. Noisy Miners; Howes et al. 2014).

The pervasive processes associated with landscape modification can become apparent many years after the actual modification occurs (e.g. fall of trees and vegetation removal), and cause adverse effects on communities and populations (i.e. extinction debt) (Ford et al. 2009). Many consequences of habitat fragmentation, such as changes in genetic, morphological or behavioural traits of species, require time to appear (Tilman et al. 1994). In contrast, the combined effects of fragmentation with climate change, human-altered disturbance regimes, species interactions and/or other drivers of population decline, may increase the rate of effects from fragmentation (Ewers and Didham 2006). Climate change can exacerbate the adverse effects associated with habitat loss and fragmentation (Mantyka-pringle 2012). Negative synergistic effects of climate change and habitat loss have the greatest effect on species in areas that are experiencing increasing maximum temperatures (Mantyka-pringle 2012) and the effects from habitat loss will likely become even more prevalent in future climates.

As with most short-term field studies (Sunnucks 2011), the temporal scale of this investigation is unlikely to have captured all elements affecting the entire population in the southern distribution of the Grey-crowned Babbler. The sampling design (Chapter 2) and analyses employed (Chapters 2-5) have revealed population trends that are typical of long-term threatening processes on species, such as disrupted dispersal patterns (Chapters 3 and 4) and gene flow decline (Chapter 4) and are indications of a likely
extinction debt for this species. The full, long-term impacts from habitat loss and fragmentation are potentially yet to be realised for the Grey-crowned Babbler. Indeed this may also be the case for a suite of other vulnerable woodland birds in the study area (e.g. Restless Flycatchers (*Myiagra inquieta*), Diamond Firetail (*Staganopleura guttata*) (Blakers *et al.* 1984; Ford 2011).

Several characteristics of landscape modification can influence the extent of genetic response in a species, such as time since fragmentation, extent of habitat loss and spatial characteristics of habitat patches (Amos *et al.* 2014; Chapters 3 and 4). Species that have experienced population declines driven by fragmentation can also be exposed to genetic bottlenecks, a process which depletes genetic diversity and increases genetic drift (Andersen *et al.* 2004; Banks *et al.* 2005; Dixo *et al.* 2009; Chapter 4). The negative effects of bottlenecks may lead to inbreeding depression, accumulation of deleterious alleles and reduced population viability (Schiegg *et al.* 2002; Harrisson *et al.* 2013). The extent of decline in genetic diversity is often correlated with the scale of the impact (e.g. the proportion of individuals lost from a population) and the recovery rate of a species, which is inevitably related to their dispersal ability in a fragmented landscape. Species that experience increased resource competition from introduced or invasive species (e.g. Noisy Miner, Clarke and Oldland 2007; European Starling, Pell 1997), can be particularly vulnerable to deleterious genetic effects (Vrijenhoek 1985). Population recovery over an extended period that is mediated by competition can exacerbate the rate of loss of rare alleles due to genetic drift (Vrijenhoek 1985). Access to non-relatives for species that are reliant on habitat cover for dispersal (e.g. Grey-
crowned Babbler), also may experience a prolonged recovery time and increase their risk to the negative consequences of genetic drift.

### 6.3 Management of a declining species in fragmented habitat at a landscape-level

This thesis has identified several genetic consequences of habitat loss and fragmentation that threaten the population viability of the Grey-crowned Babbler in the southern part of its range: i) population genetic substructure across a broad, highly fragmented, landscape; ii) declines in gene flow from an historic baseline to low contemporary gene flow rates per generation, and; iii) isolation-by-resistance detected in both sexes, but more pronounced in females, which are the more philopatric sex. These consequences, which are discussed in this thesis (Chapters 3-5), reflect reported population declines of the Grey-crowned Babbler in the study area (Robinson 2006; Radford 2008; Department of Environment and Heritage 2013). The implications of landscape-scale habitat modification on the species genetic processes seemingly exhibit a circular positive feedback system. This is demonstrated from gene flow decline which has likely instigated the population substructure (Chapter 4). Moreover, the pattern of isolation-by-resistance is a result of habitat loss and fragmentation (Chapter 5), and disrupts dispersal. This in turn determines gene flow levels between subpopulations and which subsequently influences population genetic structure (Chapters 3 and 4). Nevertheless this pervasive pattern of disruption and decline can be reversed (Bouzat et al. 1998a; Bouzat et al. 1998b).
While areas with little or no connecting vegetation often hamper an animal’s ability to move through the landscape, the provision of habitat corridors are important landscape structures that can facilitate dispersal of individuals (Beier 1993; Rosenberg et al. 1997). Major roads and highways can also pose barriers to movement (Pescador and Peris 2007). Landscape-level movement patterns observed for both sexes of the Grey-crowned Babbler were indicative of an isolation-by-resistance pattern resulting from habitat loss and fragmentation, as well as barrier effects from road presence and/or elevation level. The strong substructure detected amongst the study population highlights the need for management plans to facilitate functional connectivity between genetically isolated and/or declining subpopulations. In the west region, the Grey-crowned Babbler displayed a higher number of private alleles, while the south-east had fewer private alleles than either the west or north-east regions. Increasing gene flow amongst these three regions will likely augment genetic diversity within regions and may benefit the genetic diversity of the few groups occupying areas in-between.

Landscape modelling (Chapter 5) showed that high resistance to movement in treeless areas can be a reliable indicator for predicting dispersal direction and gene flow levels for the Grey-crowned Babbler. Overall, the results identified a lack of tree cover, major roads and/or non-habitat areas at elevation levels >300 m, as barriers to dispersal. The most likely conduits assisting gene flow were identified as connected tree cover in the forms of habitat corridors and roadside vegetation. Both barriers and conduits may be manipulated (e.g. increasing landscape tree cover levels) to facilitate genetic exchange between subpopulations at greater risk (e.g. Violet Town south and Kerang.
south/Boort), thereby enhancing the long-term presence of Grey-crowned Babbler in these landscapes. Conservation management plans in fragmented landscapes particularly, need to consider opportunities to increase and enhance landscape connectivity. Therefore, long-term conservation effort should include the identification of gaps in landscape connectivity and prioritisation of restoration works in these areas to promote genetic exchange between disconnected communities.

Much of the land in the study area is privately owned however, and as such, the capacity to link subpopulations through habitat revegetation may not always be possible. The data I have presented is highly relevant to drive revegetation programs between subpopulations that have become disconnected, but also to inform a carefully managed translocation program (Weeks et al. 2011). Consideration needs to be given to any translocation of the Grey-crowned Babbler in its southern range toward a male bias, as evidence suggests both long- and short-distance male-biased dispersal patterns in the study area. Implementation of such intervention should be urgently considered for a metapopulation that appears to be slipping out of equilibrium due to reduced functional connectivity (Volpe et al. 2014).

This study provides strong indication that historically, these (sub)populations of Grey-crowned Babbler were mobile across the study area. Although the reported gene flow has declined to such low levels, evidence of long-distance dispersal and ~12% of sampled birds having dispersed (i.e. first-generation migrants; Chapter 4) is a positive sign that ongoing dispersal is still being achieved by some individuals. The relative levels of bidirectional gene flow that were observed between regions is critical information for
translocation considerations and can assist management plans (Schwartz et al. 2007) to reflect the Grey-crowned Babbler’s historic genetic exchange amongst contemporary disparate populations. Translocation programs could potentially be implemented as an interim measure until habitat revegetation can start to provide landscape connectivity in these areas.

An unexpected result in this research was the lack of evidence for neighbouring groups influencing population dynamics and movement of individuals amongst subpopulations (Chapters 2 and 5). The lack of effect from neighbours might be a factor of survey records (Robinson unpub. data). Some data I used in landscape modelling (chapter 5) that related to extant groups, dates back 30 years and previously recorded groups may have since suffered extinction. Alternatively, no effect from neighbours on individuals dispersal may result from small sample size and hence, a lack of power to identify an effect. A more intensive sampling regime of Grey-crowned Babbler groups in areas that were not sampled may reveal different results that indicate for example, higher gene flow between study groups and currently unsampled groups and/or greater influence of isolation-by-distance at a landscape-level.

It is important then to maintain larger subpopulations and enhance their functional connectivity with other smaller and/or isolated subpopulations (i.e. maintain metapopulation processes). More stable and genetically robust subpopulations will benefit population persistence and evolutionary potential under increasing pressures from landscape modification, extreme events and climate change uncertainties (Nimmo et al. 2015). Therefore, initial strategic conservation planning should consider
implementing carefully managed, scientifically validated, translocation programs alongside revegetation efforts. An holistic approach could incorporate reintroductions of the Grey-crowned Babbler into vacant habitat areas associated with critical pathways identified in this study (e.g. tributaries of the Murray River) and target revegetation works to connect these areas with populations that have shown to be increasing (i.e. Lurg). Such actions may provide the Grey-crowned Babbler with opportunities to increase their long-term persistence and population viability through immediate potential benefits from new neighbours and increasing landscape connectivity levels in the future.

6.4 Future studies

The implications of this study are potentially applicable to other threatened species and useful for conservation management purposes. Results can be generalised directly for other species in similar situations or life-histories (e.g. cooperative-breeding avian species in modified habitats). The methods that were employed demonstrate a degree of efficacy to identifying issues as they relate to connectivity and gene flow in threatened and declining populations. In this context, broad application of these techniques provide valuable insight for threatened species. For example, a recent project on common woodland birds and their movement capacity in south-eastern Australia revealed that the study species were highly mobile across a fragmented landscape (Amos et al. 2014). A clear response to landscape connectivity levels however
was not always apparent for most of the species under study. Opportunities for a similar study incorporating this thesis’ methods but explicitly targeting a suite of threatened species known or thought to be declining due to fragmentation, would be a worthy undertaking for the conservation of threatened species and biodiversity management.

Already the ‘next-generation’ genetic research developments are becoming widely used, such as genomics, and will assist studies such as described here. The development of genomics has enabled increases in orders of magnitude in the capacity to study loci, such that, compared to the 10-20 loci typically available for current genetic research, genomics provides thousands of single nucleotide polymorphisms (Allendorf et al. 2010). Such emerging techniques that require only low sample numbers (e.g. 2-4) per population, will allow for a simpler field sampling component for ecological studies and potentially decrease resource and time requirements for large-scale studies. Future research relating to the Grey-crowned Babbler, for instance, would benefit by incorporating the use of genomics to evaluate the samples collected and incorporating genetic research previously undertaken in northern populations of the species (Blackmore et al. 2006; Kawano et al. 2007), and could potentially broaden investigations of the species’ genetic processes to a continent-scale. Indeed, because genomic studies require only a small number of samples per population, a relatively small amount of effort to strategically sample populations surrounding the subpopulations sampled here (e.g. in southern NSW; central Victoria; Australian Capital Territory) would increase our understanding of population processes and threats for this
species further.


Cushman SA, McKelvey KS, Schwartz MK (2009) Use of empirically derived source-


Edwards SV (1993b) Mitochondrial gene genealogy and gene flow among island and
mainland populations of a sedentary songbird, the Grey-crowned Babbler 

helping behaviour of the Grey-crowned Babbler, Pomatostomus temporalis. 

Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, 
Nakamura M, Nakazawa Y, Overton JM, Peterson AT, Phillips SJ, Richardson K, 
7590.04596.x.

Elkin CM, Possingham HP (2008) The role of landscape-dependent disturbance and 
doi: 10.1086/590962.


Environment Conservation Council (1997) Box-Ironbark: forests and woodlands 
investigation resources and issues report. Environment Conservation Council,
East Melbourne, Victoria.


pattern and countryside heterogeneity. Ecological Applications 18:185-196.


Kupfer JA, Malanson GP, Franklin SB (2006) Not seeing the ocean for the islands: The
mediating influence of matrix-based processes on forest fragmentation effects.


Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of
Heredity, 89, 238–247.


and Restoration 6:206-211.


Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and


Pavlova A, Amos JN, Goretskaia MI, Beme IR, Buchanan KL, Takeuchi N, Radford JQ, Sunnucks P (2012) Genes and song: Genetic and social connections in


Skroblin A, Cockburn A, Legge S (2014) The population genetics of the Western Purple-crowned Fairy-wren (Malurus coronatus coronatus), a declining riparian


Tzaros C (2001) Field surveys and population monitoring of the Grey-crowned Babbler Pomatostomus temporalis in the Loddon and Murray Valley regions, north-west Victoria. Department of Natural Resources and Environment, Melbourne,


Appendix 1

Table A1.1 Group size of the Grey-crowned Babbler and the number of groups that recorded breeding success at least once during the period June 2010 to April 2011, for study sites across the west, south-east and north-east study regions. Percentages are shown in parentheses.

<table>
<thead>
<tr>
<th>Region</th>
<th>Min</th>
<th>Group size</th>
<th>Mean</th>
<th>Total individuals</th>
<th>No. of groups</th>
<th>No. of groups detected with fledglings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>West</td>
<td>3</td>
<td>12</td>
<td>6.6</td>
<td>158</td>
<td>24</td>
<td>16 (67)</td>
</tr>
<tr>
<td>South-east</td>
<td>2</td>
<td>9</td>
<td>5.4</td>
<td>130</td>
<td>24</td>
<td>14 (52)</td>
</tr>
<tr>
<td>North-east</td>
<td>2</td>
<td>12</td>
<td>4.9</td>
<td>117</td>
<td>24</td>
<td>12 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>12</td>
<td>5.6</td>
<td>405</td>
<td>72</td>
<td>42 (58)</td>
</tr>
</tbody>
</table>
Table A1.2 Comparison between regions of habitat characteristics in Grey-crowned Babbler territories based on ANOSIM

Table shows values for: (i) west (W) versus south-east (Se) (ii) west versus north-east (Ne), (iii) south-east versus north-east; variables that contributed to 90% of the dissimilarity between regions. The largest regional variable cover in each comparison is shown in bold.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Variable</th>
<th>Mean cover (%) within region</th>
<th>West</th>
<th>South-east</th>
<th>North-east</th>
<th>mean dissimilarity</th>
<th>SD</th>
<th>contribution %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) W v Se</td>
<td>Grass (short)</td>
<td></td>
<td>31.2</td>
<td>65.2</td>
<td>12.0</td>
<td>12.0</td>
<td>1.9</td>
<td>34.4</td>
</tr>
<tr>
<td></td>
<td>Leaf litter</td>
<td></td>
<td>61.5</td>
<td>72.8</td>
<td>6.7</td>
<td>6.7</td>
<td>1.3</td>
<td>19.2</td>
</tr>
<tr>
<td>Avg. dissimilarity:</td>
<td></td>
<td></td>
<td>34.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shrub</td>
<td></td>
<td>13.6</td>
<td>9.0</td>
<td>5.3</td>
<td>5.3</td>
<td>0.8</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>Grass (tall)</td>
<td></td>
<td>2.7</td>
<td>5.8</td>
<td>1.6</td>
<td>1.6</td>
<td>1.3</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Tree (&lt;10 cm DBH)</td>
<td></td>
<td>7.2</td>
<td>9.1</td>
<td>2.9</td>
<td>2.9</td>
<td>0.9</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Tree (10-30 cm DBH)</td>
<td></td>
<td>7.8</td>
<td>9.1</td>
<td>2.3</td>
<td>2.3</td>
<td>1.3</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Lignum</td>
<td></td>
<td>4.9</td>
<td>0.0</td>
<td>1.6</td>
<td>1.6</td>
<td>0.9</td>
<td>4.4</td>
</tr>
<tr>
<td>(ii) W v Ne</td>
<td>Grass (short)</td>
<td></td>
<td>31.2</td>
<td>69.9</td>
<td>13.4</td>
<td>13.4</td>
<td>2.00</td>
<td>36.6</td>
</tr>
<tr>
<td>Avg. dissimilarity:</td>
<td></td>
<td></td>
<td>36.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf litter</td>
<td></td>
<td>61.5</td>
<td>66.8</td>
<td>6.4</td>
<td>6.4</td>
<td>1.3</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>Shrub</td>
<td></td>
<td>13.6</td>
<td>3.1</td>
<td>4.5</td>
<td>4.5</td>
<td>0.7</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>Grass (tall)</td>
<td></td>
<td>2.7</td>
<td>13.3</td>
<td>3.7</td>
<td>3.7</td>
<td>1.3</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>Tree (&lt;10 cm DBH)</td>
<td></td>
<td>7.2</td>
<td>6.9</td>
<td>2.5</td>
<td>2.5</td>
<td>0.8</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Tree (10-30 cm DBH)</td>
<td></td>
<td>7.8</td>
<td>6.4</td>
<td>2.2</td>
<td>2.2</td>
<td>1.3</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Lignum</td>
<td></td>
<td>4.9</td>
<td>0.0</td>
<td>1.6</td>
<td>1.6</td>
<td>0.9</td>
<td>4.2</td>
</tr>
<tr>
<td>(iii) Se v Ne</td>
<td>Grass (short)</td>
<td></td>
<td>65.2</td>
<td>69.7</td>
<td>7.4</td>
<td>7.4</td>
<td>1.2</td>
<td>29.9</td>
</tr>
<tr>
<td>Avg. dissimilarity:</td>
<td></td>
<td></td>
<td>24.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf litter</td>
<td></td>
<td>72.8</td>
<td>66.8</td>
<td>5.4</td>
<td>5.4</td>
<td>1.2</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>Shrub</td>
<td></td>
<td>9.0</td>
<td>3.1</td>
<td>2.7</td>
<td>2.7</td>
<td>0.5</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>Grass (tall)</td>
<td></td>
<td>5.8</td>
<td>13.3</td>
<td>2.8</td>
<td>2.8</td>
<td>1.1</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Tree (&lt;10 cm DBH)</td>
<td></td>
<td>9.1</td>
<td>6.9</td>
<td>2.4</td>
<td>2.4</td>
<td>0.8</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>Tree (10-30 cm DBH)</td>
<td></td>
<td>9.1</td>
<td>6.4</td>
<td>1.9</td>
<td>1.9</td>
<td>1.3</td>
<td>7.8</td>
</tr>
</tbody>
</table>
Table A1.3 Within-region similarities in habitat characteristics of Grey-crowned Babbler territories.
The table shows variables that contributed to 90% of similarity within regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Habitat variable</th>
<th>Average</th>
<th>Average</th>
<th>Similarity</th>
<th>Contribution</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>abundance</td>
<td>similarity</td>
<td>SD</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>West</td>
<td>Leaf litter (%)</td>
<td>61.50</td>
<td>37.74</td>
<td>4.13</td>
<td>57.36</td>
<td>57.36</td>
</tr>
<tr>
<td>Avg. similarity: 65.81</td>
<td>Grass &lt;10 cm ht (%)</td>
<td>31.24</td>
<td>16.02</td>
<td>1.91</td>
<td>24.35</td>
<td>81.71</td>
</tr>
<tr>
<td></td>
<td>Tree 10 - 30 cm DBH</td>
<td>7.84</td>
<td>2.75</td>
<td>1.02</td>
<td>4.17</td>
<td>85.88</td>
</tr>
<tr>
<td></td>
<td>Shrub &gt;1 m ht</td>
<td>13.63</td>
<td>2.32</td>
<td>0.41</td>
<td>3.53</td>
<td>89.41</td>
</tr>
<tr>
<td></td>
<td>Tree &lt;10 cm DBH</td>
<td>7.15</td>
<td>1.94</td>
<td>0.85</td>
<td>2.96</td>
<td>92.36</td>
</tr>
<tr>
<td>North-east</td>
<td>Grass &lt;10 cm ht (%)</td>
<td>69.86</td>
<td>33.09</td>
<td>3.21</td>
<td>43.06</td>
<td>43.06</td>
</tr>
<tr>
<td>Avg. similarity: 76.84</td>
<td>Leaf litter (%)</td>
<td>66.84</td>
<td>32.91</td>
<td>4.53</td>
<td>42.83</td>
<td>85.89</td>
</tr>
<tr>
<td></td>
<td>Grass 40 cm ht (%)</td>
<td>13.28</td>
<td>4.69</td>
<td>1.49</td>
<td>6.11</td>
<td>92.00</td>
</tr>
<tr>
<td>South-east</td>
<td>Leaf litter (%)</td>
<td>72.80</td>
<td>36.13</td>
<td>5.15</td>
<td>48.11</td>
<td>48.11</td>
</tr>
<tr>
<td>Avg. similarity: 75.10</td>
<td>Grass &lt;10 cm ht (%)</td>
<td>65.18</td>
<td>28.46</td>
<td>3.00</td>
<td>37.90</td>
<td>86.00</td>
</tr>
<tr>
<td></td>
<td>Tree 10 - 30 cm DBH</td>
<td>9.10</td>
<td>3.41</td>
<td>1.67</td>
<td>4.55</td>
<td>90.55</td>
</tr>
</tbody>
</table>
Figure A1.1 Non-metric multi-dimensional scaling ordination of study sites within three regions based on the differences in habitat attributes.
West region (black triangles); south-east region (grey circles); north-east region (white diamonds)
Appendix 2

Table A2.1 Global characterisation of 13 microsatellite loci of the Grey-crowned Babbler within its southern-most distribution.

Values show: number of samples amplified for the locus ($N_o$); number of alleles ($A$); observed heterozygosity ($H_o$); expected heterozygosity ($H_e$). Averages are shown in the last row, and loci that deviated from Hardy-Weinberg equilibrium within a genetic cluster are marked with an asterisk.

<table>
<thead>
<tr>
<th>Locus</th>
<th>$N_o$</th>
<th>$A$</th>
<th>$H_o$</th>
<th>$H_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pte17</td>
<td>135</td>
<td>4</td>
<td>0.50</td>
<td>0.46</td>
</tr>
<tr>
<td>Pte24</td>
<td>135</td>
<td>4</td>
<td>0.51</td>
<td>0.49</td>
</tr>
<tr>
<td>Pte42</td>
<td>135</td>
<td>9</td>
<td>0.53</td>
<td>0.57</td>
</tr>
<tr>
<td>Pte47*</td>
<td>135</td>
<td>5</td>
<td>0.59</td>
<td>0.64</td>
</tr>
<tr>
<td>Pte48</td>
<td>135</td>
<td>4</td>
<td>0.59</td>
<td>0.64</td>
</tr>
<tr>
<td>Pte50*</td>
<td>135</td>
<td>11</td>
<td>0.81</td>
<td>0.86</td>
</tr>
<tr>
<td>Pte101</td>
<td>135</td>
<td>15</td>
<td>0.84</td>
<td>0.88</td>
</tr>
<tr>
<td>Pte102</td>
<td>134</td>
<td>19</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Pte103</td>
<td>135</td>
<td>13</td>
<td>0.88</td>
<td>0.89</td>
</tr>
<tr>
<td>Pte105</td>
<td>135</td>
<td>7</td>
<td>0.80</td>
<td>0.81</td>
</tr>
<tr>
<td>Pte106</td>
<td>135</td>
<td>8</td>
<td>0.81</td>
<td>0.82</td>
</tr>
<tr>
<td>Pte108</td>
<td>135</td>
<td>13</td>
<td>0.80</td>
<td>0.87</td>
</tr>
<tr>
<td>Pte109*</td>
<td>135</td>
<td>31</td>
<td>0.88</td>
<td>0.93</td>
</tr>
<tr>
<td>Mean</td>
<td>135</td>
<td>11</td>
<td>0.73</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Table A2.2 FRAGSTATS landscape dispersed and aggregated statistics for tree cover in the east and west study regions.
Indices of tree cover aggregation calculated from 100 m pixel tree cover raster for the combined east regions and the west region in FRAGSTATS v 4 (McGarigal et al. 2012) indicating whether the indices show support for the east regions having higher levels of dispersed tree cover than the west region.

<table>
<thead>
<tr>
<th>FRAGSTATS metric</th>
<th>Region</th>
<th>Support for tree cover being more dispersed in the east</th>
<th>Description/comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>East</td>
<td>West</td>
<td></td>
</tr>
<tr>
<td>Number of patches</td>
<td>49739</td>
<td>23650</td>
<td>Y</td>
</tr>
<tr>
<td>Patch Density</td>
<td>1.62</td>
<td>0.77</td>
<td>Y</td>
</tr>
<tr>
<td>Landscape shape index</td>
<td>178.62</td>
<td>118.69</td>
<td>Y</td>
</tr>
<tr>
<td>Mean patch area</td>
<td>10.62</td>
<td>13.65</td>
<td>Y</td>
</tr>
<tr>
<td>Euclidean nearest neighbour patch distance</td>
<td>304.50</td>
<td>366.18</td>
<td>Y</td>
</tr>
<tr>
<td>Clumpy</td>
<td>0.70</td>
<td>0.77</td>
<td>Y</td>
</tr>
<tr>
<td>Percentage of like adjacencies</td>
<td>75.41</td>
<td>79.10</td>
<td>Y</td>
</tr>
<tr>
<td>Cohesion</td>
<td>97.83</td>
<td>98.00</td>
<td>Y (marginal)</td>
</tr>
<tr>
<td>Aggregation index (AI)</td>
<td>75.51</td>
<td>79.24</td>
<td>Y</td>
</tr>
<tr>
<td>Normalised landscape shape index</td>
<td>0.24</td>
<td>0.21</td>
<td>Y</td>
</tr>
</tbody>
</table>

total number of patches greater in east number of patches/ha
west has lower edge density or total edge, standardised for landscape size
Larger (aggregated) patches in west east patches closer together
west is marginally more clumpy i.e. contagion is higher, for example, when a single class occupies a very large percentage of the landscape, and is inversely related to edge density
west has greater aggregation of patch types e.g. larger patches with compact shapes
west has slightly more patch cohesion i.e. as the value increases the patch type becomes more clumped or aggregated in its distribution; hence, more physically connected/larger patches
west is slightly less checkerboard landscape than east i.e. it has less patch edges