The Breeding Ecology and Mating System of the Bellbird (*Anthornis melanura*) on Tiritiri Matangi Island.

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Abstract

A large population of Bellbirds were individually banded and monitored on Tiritiri Matangi Island in 2005 and 2006. The main aim of this research was to provide baseline breeding data from a large, stable population of Bellbirds reminiscent of pre-colonisation New Zealand. Nesting observations indicated that Bellbirds preferentially nest in Cabbage Trees on the island. Nest success was similar to recent values detected for other open nesting passerines found on the island, and has not changed since the study by Anderson & Craig (2003) in undertaken 1979. This is interesting considering that predation pressures would have been significantly alleviated since the eradication of Kiore in 1993. Breeding was found to be highly asynchronous within neighbouring territories in both 2005 and 2006. The majority of social bonds were recorded as monogamous, similar to past findings; however this research reported one case of polygynandry. In addition, regular extra pair male visits to other nests were recorded, as well as the occurrence of extra pair copulations. Parental care was undertaken by both sexes; however was largely unequal in that the female invested more in nest attendance than the male. The inequality in parental care, as well as the observed extra pair social behaviours, led to doubts over the current certainty of monogamy as the mating system in this species. The genetic analysis of paternity revealed that both males and females engage in mixed mating strategies, with 81% of offspring a result of extra pair paternity. This represents one of the highest levels of promiscuity recorded in passerines to date. The high level of sexual dimorphism coupled with the high level of promiscuity indicates the importance of genetic evidence for conclusions regarding mating systems; especially in the honeyeater species that show sexual dimorphism and hence intense sexual selection.

Male Bellbird (*Anthornis melanura melanura*).
Photos: Jan Doak, 2007.

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Wet male Bellbird.
Photo: Jan Doak, 2007.

Table of Contents

ABSTRACT ii

ACKNOWLEDGEMENTS iv

TABLE OF CONTENTS vii

LIST OF FIGURES xi

LIST OF TABLES xiii

CHAPTER ONE: GENERAL INTRODUCTION 1

1.1. REPRODUCTIVE BIOLOGY AND CONSERVATION. 2

1.2. NEW ZEALAND CONSERVATION 3

1.3. STUDY SPECIES: THE BELLBIRD (*Anthornis melanura*). 3

1.4. STATUS OF CURRENT KNOWLEDGE OF BELLBIRDS. 8

1.5. GAPS IN KNOWLEDGE 9

1.6. STUDY SITE 10

1.7. BELLBIRDS ON TIRITIRI MATANGI ISLAND 11

1.8. AIMS AND STRUCTURE OF THIS STUDY. 15

CHAPTER TWO: PHYSICAL BREEDING PARAMETERS 16

2.1. ABSTRACT 17

2.2. INTRODUCTION 18

2.2.1. Objectives 19

2.3. METHODOLOGY 19

2.3.1. Bird capture 20

2.3.2. Banding and Measurements 20

2.3.3. Blood Sampling 21

2.3.4. Nesting biology 22

2.3.5. Breeding biology 23
2.3.5.1. Chick growth 23
2.3.5.2. Breeding Synchrony 25
2.3.5.3. Nest success 26

2.3.6. Primary sex ratios of clutches 26

2.4. RESULTS 27

2.4.1. Banding and Measurements 27

2.4.2. Timing of breeding 30

2.4.3. Breeding Synchrony 30

2.4.4. Clutch size, incubation length and nestling dependence 32
2.4.4.1. Nestling growth 33

2.4.5. Nesting Biology 35
2.4.5.1. Nest dimensions and lining 35
2.4.5.2. Nest tree preferences 35
2.4.5.3. Nest tree preference and nest success 36
2.4.5.4. Nest cover 37
2.4.5.5. Nest Success 39

2.4.6. Primary Sex Ratios 40

2.5. DISCUSSION 41

2.5.1. Adult Measurements 41

2.5.2. Nesting and Breeding Biology 41

2.5.3. Primary Sex Ratios 46

2.5.4. Recommendations for future management 48

2.6. CONCLUSION 49

CHAPTER THREE: SOCIAL BREEDING PARAMETERS 50

3.1. ABSTRACT 51

3.2. INTRODUCTION 51

3.2.1. Objectives 58

3.3. METHODOLOGY 59

3.3.1. Nest observations 59

3.3.2. Statistical Analysis 61

3.4. RESULTS 62

3.4.1. Parental Care 62
3.4.1.1. The effect of brood size and sex ratio 62
3.4.1.2. Differential investment between parents 64
3.4.1.3. Individual Variation 68

3.4.2. Social interactions 69

3.5. DISCUSSION 72

3.5.1. Parental Care 72

3.5.2. Social interactions 76

3.6. CONCLUSION 81

CHAPTER FOUR: GENETIC INVESTIGATION OF PATERNITY. 82

4.1. ABSTRACT 83

4.2. INTRODUCTION 83

4.2.1. Objectives 88

4.3. METHODOLOGY 89

4.3.1. DNA preparation 89

4.3.2. Quantification of digested DNA 89

4.3.3. DNA analysis by Southern blotting 90

4.3.4. Data Analysis 92

4.4. RESULTS 94

4.4.1. Bandsharing patterns 94

4.4.2. Paternity Assignment 97

4.4.3. Special Cases 99

4.4.4. Correlations with paternity 101

4.5. DISCUSSION 102

4.6. CONCLUSION 108

CHAPTER FIVE: CONCLUSIONS 109

5.1. OUTLINE OF THIS CHAPTER: 110

5.2. MELIPHAGIDAE PHYLOGENY AND BACKGROUND 110

5.3. BEHAVIOURAL SIMILARITIES OF THE NEW ZEALAND BELLBIRD AND THE AUSTRALIAN HONEYEATERS 112

5.4. SIMILARITY BETWEEN CHARACTERISTIC BEHAVIOURS OF THE BELLBIRD AND AUSTRALIAN HONEYEATERS 113

5.5. SEXUAL DIMORPHISM AND MATING SYSTEMS IN THE MELIPHAGIDAE. 118

5.6. THE GENETIC MATING SYSTEM OF THE BELLBIRD IN RELATION TO CURRENT KNOWLEDGE OF THE AUSTRALIAN HONEYEATERS. 120

5.7. THE NEW ZEALAND BELLBIRD AS A MODEL SPECIES FOR UNDERSTANDING BREEDING BIOLOGY AND MATING SYSTEMS. 121

5.8. DIRECTIONS FOR FUTURE RESEARCH: 123

5.9. MANAGEMENT RECOMMENDATIONS: 124

REFERENCES 125

APPENDIX I 142

APPENDIX II 144

APPENDIX III 149

List of Figures

Page No.

Chapter 1

1.1 Detailed photographs showing sexual dimorphism between female and male Bellbirds. 4

1.2 Current distribution patterns of Bellbird in Northern New Zealand. 6

1.3 Tiritiri Matangi Island. 11

1.4 Sugar water feeders on Tiritiri. 13

1.5 Area of focus for this study on Tiritiri Matangi Island. 14

Chapter 2

2.1 Photographic guide of Bellbird nestlings of known age up to day 11 of development. 24

2.2 Frequency of adult male and female body measurements on Tiritiri. 29

2.3 Overlap in egg laying dates from the start of the breeding season in 2005 and 2006. 31

2.4 Variation in egg laying dates per season. 32

2.5 Logistic growth curves for Bellbird Nestlings. 34

2.6 Tree species selected for nest sites by Bellbirds on Tiritiri Matangi Island. 36

2.7 Number of successful (fledging at least one young) and unsuccessful Nests according to nest species preference. 37

2.8 Examples of nest cover over Bellbird nests on Tiritiri in 2005 and 2006. 38

Chapter 3

3.1 Average number of nest visits per hour at each nest stage for males and females combined. 63

3.2 Average number of visits by male and female Bellbirds per hour. 66

3.3 Average length of time on nest for each visit per hour for chicks and fledging stages. 66

3.4 Average length between nest visits per hour for male and female Bellbirds at each nest stage. 67

3.5 Association between frequency of male visits per hour and female visits per hour during the chick stage. 67

3.6 Deviation from 'best' female and male in percentage of nest observation time allocated to nesting behaviour. 68

3.7 Frequency of male and female chases per hour at each nest stage, showing the corresponding rate of extra-pair male (EPM) visits to nests at those stages. 70

3.8 Territories of Bellbirds within the study site. 71

Chapter 4

4.1 Autoradiograph showing individual 'fingerprints' of bands. 91

4.2 Relationship between the band-sharing coefficient and number of novel bands for each nestling compared with the putative father and the mother. 96

4.3 Frequency distributions of novel bands present in offspring for Jeffreys 33.15 probe, CA probe and both probes combined. 98

Chapter 5

5.1 Principal components analysis of physical breeding parameters of the Australian and New Zealand Meliphagidae. 114

5.2 Male Bellbird posturing and displaying in a 'coroboree' aggregation. 118

5.2 Distribution of current mating system classification and level of sexual dimorphism for the Australian and New Zealand honeyeaters. 119

List of Tables

Page No.

Chapter 2

2.1 Inter-sexual size differences in adult Bellbirds showing mean for body measurements with the corresponding student's t-values and level of significance. 28

2.2 Estimates of nest success of Bellbird breeding on Tiritiri using Apparent, Mayfield and Stanley's estimates. 40

Chapter 3

3.1 Definitions of documented behaviours during Bellbird nest watches. 60

3.2 Number of nest visits per hour at each nest stage. 65

3.2 The hypothesis formulated for the role of female song and their expected behaviours with the current evidence for the role of female song. 79

Chapter 4

4.1 Bandsharing coefficients for adults within the Bush 1 Tiritiri Matangi Island Bellbird population. 97

CHAPTER ONE: General Introduction

Male Bellbird in flight.
Photo: Weihong Ji, 2005

1.1. Reproductive biology and conservation.

The decline of species throughout the world, in particular birds, has attracted attention from conservationists throughout time. Understanding the causes of decline is usually limited by lack of knowledge of the life history and ecology of a species (Geering & French, 1998). Conservation Science is often referred to as a "crisis discipline" (Soulé, 1990), where actions taken to recover species are often engaged too late and in a hasty fashion. The ultimate situation would be to study a species in detail, so as to understand the limiting factors on a population before crisis conservation is required.

The understanding of a species' mating system is essential since mate choice has large implications for conservation (Quader, 2005). This is because the reproductive behaviour of a species is directly related to its population dynamics, and is thus the most relevant aspect of a species' ecology to conservation management. Due to random fluctuations in the numbers of males and females (demographic stochasticity), the social mating system can directly impact on the number of individuals that reproduce, affecting the likelihood of extinction (Legendre, Clobert, Moller & Sorci, 1999). Monogamous mating systems have been shown to have a higher extinction risk than polygamous mating systems (Legendre *et al.*, 1999; Saether, Engen, Lande, Moller, Bensch, Hasselquist, Beier & Leisler, 2004).

Biased reproductive success of certain individuals can limit populations by reducing genetic variation (Lacy, 1987). The loss of genetic variation can affect population fitness and viability leading to increased risk of extinction (Caughley, 1994). The understanding of genetic variation within a population, as well as the variation in genetic contribution of individuals to future generations, is essential for conservation and management of that species.

1.2. New Zealand Conservation

Populations of New Zealand birds have declined drastically since European settlement (Turbott, 1957) through the introduction of mammalian predators as well as habitat loss and modification (Diamond & Veitch, 1981). Offshore islands have been established as refuges for many native species and have become essential in saving endangered New Zealand species (Mortimer, Sharp & Craig, 1996). An example of a species affected by European colonisation is the New Zealand Bellbird (*Anthornis melanura*). Although common in some areas of New Zealand, Bellbird populations went through dramatic declines with imminent extinction feared by early ornithologists (e.g.: W.Buller).

1.3. Study Species: The Bellbird (*Anthornis melanura*).

The New Zealand Bellbird (*Anthornis melanura*) is a medium sized sexually dimorphic honeyeater (Figure 1.1), from the Australo-Papuan family Meliphagidae. It is genetically more closely related to the Tui (Driskell, Christidis, Gill, Boles, Barker & Longmore, 2007) than to any of the Australian Meliphagidae.

(A)

Female Bellbird head profile.
Photo: Dianne Brunton, 2006.

(B)

Male Bellbird.
Photo: Jan Doak, 2007

Figure 1.1: Detailed photographs showing sexual dimorphism between female (A) and male (B) Bellbirds.

The Bellbird is endemic to the islands of New Zealand with four subspecies having been identified. The most common and widely spread subspecies of Bellbird is *A. melanura melanura* which occurs throughout the North and South Island of New Zealand, but is absent from the mainland north of Auckland (Higgins & Steele, 2001). However, populations are present on the offshore islands off Auckland and Northland. Other subspecies include Poor Knights Islands subspecies, *A. melanura oneho*, which breeds only on the Poor Knights Islands off the coast of Tutukaka in Northland and has limited movement to the mainland (Heather & Robertson, 1996). The Three Kings Islands Bellbird, *A. melanura obscura*, exists solely on the Three Kings Islands and the Chatham Island Bellbird, *A. melanura melanocephala*, existed solely on the Chatham Islands until it became extinct in 1906 (Heather & Robertson, 1996); (Higgins & Steele, 2001).

Early ornithological reports from the time of European settlement of New Zealand suggest that Bellbirds were abundant throughout New Zealand, including Northland and Auckland, until the 1860's when numbers decreased rapidly (Buller, 1873). The decline in numbers was not limited to north of Auckland. Noticeable decreases in encounters with this once common bird were noted throughout the North Island (Turbott, 1953) along with subsequent increases in the early 1900's (Myers, 1923). Populations around Christchurch were virtually extinct by 1900, but the numbers slowly and consistently increased from this point until 1930 (Higgins & Steele, 2001). This indicates that population recovery from local extinction is possible in this species. However, Bellbirds have failed to recolonise Northland in over 100 years with only sporadic sightings in areas close to the offshore islands they inhabit (these include Arid Island, Hen &

Chicken Islands, Little Barrier Island, Poor Knights Islands, Tiritiri Matangi Island and Mokohinau Islands) (Craig & Douglas, 1984). The current distribution of Bellbirds north of Auckland is presented in Figure 1.2.

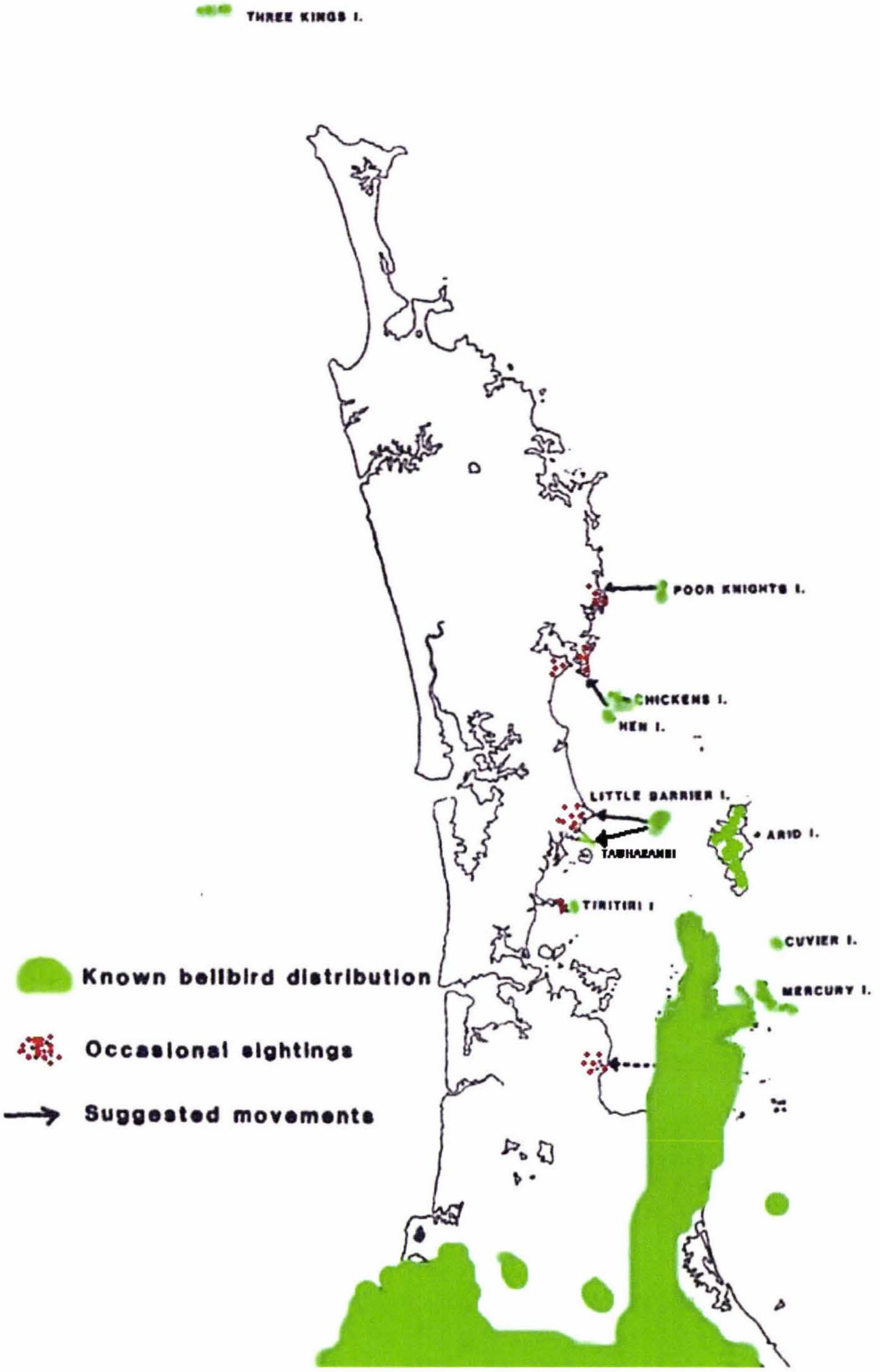


Figure 1.2: Current distribution patterns of Bellbird in northern New Zealand. Current known distributions, including the newly founded population at Tawharanui as well as occasional reported sighting of Bellbirds are shown. Map adapted from (Craig & Douglas, 1984).

There have been many debates and hypotheses over the causes for the declines, including predation, habitat loss and resource depletion. However the decline was more rapid in the North Island than the South Island despite introduced mammalian predators, bush modification and competition (with introduced honeybees) occurring throughout (Turbott, 1953). Although all of these factors would have certainly aided in the decline of bird numbers, alternate pressures on the Northland populations should be considered.

One such alternative pressure may have been an introduced disease. For example, the extinction of many of the Hawaiian Drepanididae, where significant population declines occurred in virtually undisturbed areas of forest (Warner, 1968), has been attributed, in great part, to avian malaria (*Plasmodium spp.*). In that instance, the introduction of a disease vector (the night-flying mosquito *Culex pipiens fatigans*) shortly before these population crashes is thought to have aided in the spread of avian malaria from migratory birds (Warner, 1968). Research into avian malaria within other regions of the Pacific has produced varying results. For instance, no occurrence was found in the Cook Islands (Steadman, Greiner & Wood, 1990), but Samoa has been found to hold a high prevalence of malaria, occurring at high infection rates, within native bird populations (Jarvi, Farias, Baker, Freifed, Baker, Van Gelder, Massey & Atkinson, 2003). It has also been suggested that this latter malaria is likely to be native to Samoa (Jarvi *et al.*, 2003). In contrast, a number of different avian malaria species have been found in a range of Australian bird populations (e.g.: Peirce, Lederer, Adlard & O'Donoghue, 2004).

Bird species that are initially vulnerable to malaria can develop immunity, as recently demonstrated in Hawaii (Woodworth, Atkinson, LaPointe, Hart, Speigel, Tweed, Henneman, LeBrun, Denette, DeMots, Kozar, Triglia, Lease, Gregor, Smith & Duffy, 2005). Avian malaria (*Plasmodium spp.*) blood parasites have been detected at high levels in Bellbirds on Tiritiri Matangi Island, but appear to have limited negative affects on the population (Barraclough, Cope, Pierce & Brunton, *in review*). These parasites have also been found within the Little Barrier Island population (J.Ewen, *unpub.data*). It is possible that this avian malaria (or another disease) may have previously decimated Bellbird populations, but these have recovered through the development of immunogenic responses.

1.4. Status of current knowledge of Bellbirds.

Bellbirds remain one of three bird species still present on the mainland able to aid in pollination of New Zealand forest flora (Clout & Hay, 1989). They feed on nectar, fruit, insects and insect by-products such as honeydew (Higgins & Steele, 2001) and play an essential role in seed dispersal and germination of New Zealand flora, such as the endangered mistletoes (Loranthaceae) (Ladley & Kelly, 1996).

Bellbirds are extremely aggressive, as is the pattern throughout the Meliphagidae family (Pyke, Christy & Major, 1996). Dominance hierarchies exist among the New Zealand nectivorous birds, with Tui (*Prosthemadera novaeseelandiae*) dominating Bellbird, which in turn dominate Hihi (*Notiomystis cincta*) (Craig, 1985). Bellbirds also exhibit complex social dominance hierarchies between individuals, with males dominating females which dominate juveniles (Craig & Douglas, 1986). However, a resident female may dominate a non-resident male at food sources within her territory (Higgins & Steele, 2001). Bellbirds are unusual among passerines in that female

Bellbirds sing throughout the breeding season and throughout the year (Brunton & Li, 2006).

Breeding bonds in Bellbirds have been documented as monogamous (Higgins & Steele, 2001; Anderson & Craig, 2003) with no direct evidence for divorce or infidelity.

1.5. Gaps in knowledge

Relatively little is known about the breeding ecology of Bellbirds. A review of published literature shows that foraging and aggression are the most widely studied topics involving Bellbirds. Studies on the breeding ecology of Bellbirds are limited to a small study on Tiritiri Matangi Island with limited sample sizes (Anderson & Craig, 2003) and one study on the Poor Knights Islands subspecies (Sagar, 1985).

Virtually nothing is known on individual reproductive success, mate choice or sex ratios of populations of Bellbirds. Information on social dynamics and nesting activities are limited to small sample sizes in populations with low numbers of banded individuals (e.g.: Anderson & Craig, 2003). There have been three studies investigating song in Bellbirds (Li, 2002; Brunton & Li, 2006; Brunton, Evans, Ji & Cope, *in review*), however the role of female song and consequences of male song are yet to be understood. There is no information regarding the genetic structure, inbreeding levels or paternity in populations of Bellbirds. A classic experiment by Spielman *et al.*, (2004) showed that inbred populations of *Drosophila* were more susceptible to disease than regular outbred populations. Due to the potential bottleneck that this population was subject to, the levels of inbreeding in this highly dense, closed island population warrants further investigation (see Section 1.7).

1.6. Study site

Tiritiri Matangi Island (Tiritiri) is situated 28 kilometres (km) northeast of Auckland and 3km east of the Whangaparaoa Peninsula in the Hauraki Gulf, Auckland, New Zealand. The island is 220ha in size and consists of remnants of broadleaf vegetation, grassland and areas of re-vegetated native trees undertaken as part of a restoration programme (Mitchell, 1985) (see Figure 1.3). It is an open sanctuary for conservation, public access and scientific study and has had a protected reserve status since 1980 (Galbraith & Hayson, 1995). Stock were removed from the island in 1970 and the only mammalian predator to have been recorded on Tiritiri, the Polynesian rat *Rattus pacificus* (Kiore), was eradicated in 1992 by aerial poisoning (Graham & Veitch, 2002). A series of translocations of endangered species has established the island as an important refuge for conservation (Galbraith & Hayson, 1995).

Figure 1. 3: Tiritiri Matangi Island (Google Earth Digital Globe ©).

1.7. Bellbirds on Tiritiri Matangi Island

A remnant population of Bellbirds (*Anthornis melanura melanura*) was known to be present on Tiritiri previous to any reclamation of the island for conservation. Records from the Ornithological Society report that 24 individual Bellbirds resided on Tiritiri Matangi Island in 1969 (Rimmer, 2004).

The placement of sugar water feeders (see Figure 1.4) throughout the island for management of Hihi, as well as the successful revegetation programme, has resulted in the numbers of Bellbirds increasing dramatically in the last 30 years (Veitch, Graham & Graham, *pers.comm*). The population can now be considered high density with breeding territories of $100m^2$ (estimate taken from observations during this study). The regenerating vegetation and high density of Bellbirds make Tiritiri an ideal site to study Bellbirds.

In order to gain as much information on the nesting, breeding and social systems of Bellbirds on Tiritiri, efforts were concentrated in one section of the island (see Figure 1.5). The "Bush 1" area of Tiritiri is one of the largest areas of remnant broadleaf native forest on the island, which consists of a large concentration of artificial feeders.

Recent findings have detected an extremely high prevalence of avian malaria (*Plasmodium spp.*) in this population of Bellbirds (Barraclough *et al.*, *in review*). However, the large population size of Bellbirds on Tiritiri as well as no physical size differences between infected and uninfected individuals, suggests that the prevalence of this pathogen has limited affect on this population (Barraclough *et al.*, *in review*).

(A)

Sugar water feeder station.
Photo: Taneal Cope, 2006

(B)

Male Bellbirds at sugar water feeder.
Photo: Weihong Ji, 2006.

Figure 1. 4: Sugar water feeders on Tiritiri in a wooden cage (A) and plastic mesh (B).

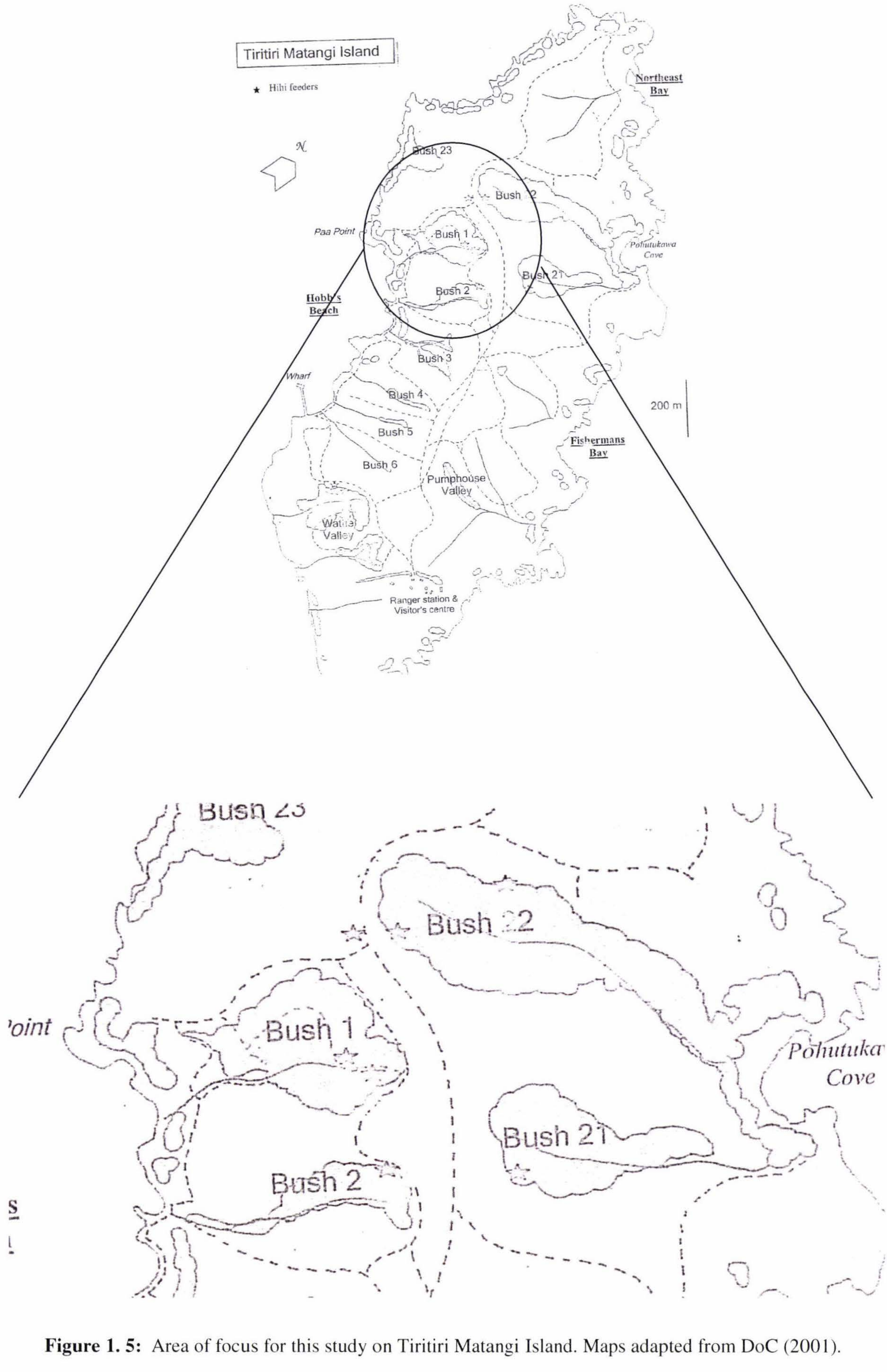


Figure 1. 5: Area of focus for this study on Tiritiri Matangi Island. Maps adapted from DoC (2001).

1.8. Aims and structure of this study.

The primary aim of this study is to provide a baseline of breeding information on Bellbirds in a large, stable population representative of pre-colonisation New Zealand.

Chapter Two aims to detail the physical aspects of the breeding ecology of Bellbirds on Tiritiri including nest site characteristics and nest success. The physical breeding biology of Bellbirds is analysed in terms of clutch size, nestling growth and dependence, and primary sex ratios of nests.

Chapter Three describes the social breeding parameters in Bellbird ecology and aims to document differences in male and female contributions to nesting. Variation between sexes and individuals in parental investment is explored. In addition to this, territorial behaviour of male and female Bellbirds is documented and related to factors including proximity to artificial feeders and numbers of adjoining neighbours.

Chapter Four analyses the occurrence of extra pair paternity in Bellbird clutches. It aims to conclude reproductive tactics of males and females through the proportion of young that are sired by males outside of the pair bond. In addition to this, levels of relatedness between individuals are explored to investigate levels of inbreeding and patterns of natal philopatry.

Chapter Five aims to relate the findings of this research to current knowledge of honeyeaters. Analysis is made to determine the most similar species to the Bellbird based on physical breeding parameters, as well as compare social behaviours to those found in the Australian honeyeaters. The result from the paternity analysis in this study is compared to those from the Australian honeyeaters and the consequences of these results implied for other sexually dimorphic honeyeaters.

CHAPTER TWO: Physical Breeding Parameters.

Interior cup of Bellbird nest
Photo: Taneal Cope, 2006

2.1. Abstract

The understanding of a species reproductive ecology is central to conservation management of that species. Reproduction directly affects population growth and understanding these factors in a large population resembling pre-colonisation New Zealand, is a rare occurrence. The breeding ecology of Bellbirds on Tiritiri was studied in 2005 and 2006 to determine the physical aspects of nesting in this species. Nesting started significantly later in 2006 than it had in 2005. However, asynchronous breeding was found throughout both seasons. Nests were primarily built in well concealed areas, in particular under the hanging fronds of Cabbage Trees. Clutch size was slightly higher than that found in a study of Bellbirds on Tiritiri in 1979. Interestingly, Bellbird nest success has not increased since this time despite the eradication of the only mammalian predator on the island, approximately 15 years ago. Incubation period was similar to that documented previously for Bellbirds; however, nestling dependence was found to be considerably longer in this study. Differences between males and females in body measurements illustrated the degree of sexual dimorphism in this species. Offspring showed a logistic pattern of growth, as is common in passerines and previously detected for Bellbirds. Male offspring grew larger and faster than females, indicating that they are potentially more costly to raise. Primary sex ratios of clutches did not vary between seasons or between first and second clutches. These results form a baseline of breeding ecology data from a large, individually banded population of Bellbirds that will aid future research on this species.

2.2. Introduction

The conservation and management of bird species is dependent on ecological knowledge of that species (Jones, 2004). Bellbirds currently exist throughout mainland New Zealand, except Northland where they have been locally extinct since 1860 (Buller, 1873; Turbot, 1983; Craig, 1984). However, the current trend of increasing numbers and occurrence of introduced mammals (King, C.M., 1990) and nectar competitors (e.g.: Starlings, *Sturnus vulgaris*) may influence the future persistence of these honeyeaters. Attempts to translocate this species to mainland sites and island refuges in northland have all failed (Craig & Douglas, 1984; Lee, 2005).

In general, it has been proposed that failures to restore populations of threatened species are in general due to insufficient knowledge of the ecological requirements of that species (Jones, 2004). Knowledge of the life history, ecology, distribution, and population size of a species is essential for subsequent conservation and management practices (Jones, 2004). This study utilises the rare opportunity to study a large, stable Bellbird population that is likely to be indicative of pre-settlement New Zealand. The aim of this research is to advance baseline ecological knowledge of the New Zealand Bellbird to assist in future management and conservation of this species. An important aspect of breeding is nest survival, and estimating the variability of nest success is important for management and conservation when assessing the vulnerabilities of a particular species. The understanding of species' nest vulnerability in a stable mammalian-predator-free environment in New Zealand is not only a rare occurrence but is useful in further understanding the impacts of introduced predators on productivity.

2.2.1. Objectives

The primary goal of this chapter is to present a detailed account of the general nesting biology of Bellbirds on Tiritiri, in particular to describe the physical aspects of the breeding ecology of this large, stable population.

Specific objectives are:

1. Describe the differences in individual measurements for adult males and females in a large banded population.
2. Describe nest characteristics including size, lining, percentage cover, height, and tree species.
3. Evaluate nest success of the focal population and compare to past studies of Tiritiri Bellbirds.
4. Describe the timing, length and synchrony of breeding within the focal population.
5. Analyse the primary sex ratios of clutches between years.

2.3. Methodology

This research was conducted with permission from the Department of Conservation under permit AK-14936-FAU (See Appendix I). Bird handling and collection of samples were undertaken with approval from the Animal Ethics Committee of Massey University (Permit Number 05/117).

The large population of Bellbirds on Tiritiri were intensively monitored from August 2005 to January 2006; and from September 2006 to December 2006.

2.3.1. Bird capture

A variety of methods were used to capture the Bellbirds. Mist nets were primarily used to capture adult birds. These were placed within territories or along regular flight paths throughout the year to target unbanded individuals from pairs. Artificial sugar feeders placed in wood/mesh cages, as part of the management of Hihi on Tiritiri, were also used to catch adult birds. Bellbirds regularly use and dominate these artificial food resources and specific individuals were targeted at feeders by using the entrance to the cage as a spring-trap.

Caught birds were handled carefully and swiftly to avoid risks and reduce stress. Efforts to capture adult birds were concentrated before the breeding season so as to reduce the impact on egg-carrying females or on pair bonds.

2.3.2. Banding and Measurements

Once caught, individual males were banded with a unique combination of two to three colour bands and a metal "C" sized band and females were banded with "B" sized bands (see Section 2.5.4). Once banded, measurements were taken and recorded for each individual. Bird weight was measured using a 100 g spring balance Pesola ® scale and recorded to the nearest 0.5 g. Wing measures were taken using a standard stopped wing rule (to the nearest 0.5 mm) from the carpal joint to the tip of the longest primary feather (as in Gosler, 1994). Tail feather measurements (to the nearest 0.5 mm) were taken using a standard stopped wing rule placed on top of the tail, stopped end on the base of the tail and read with the tail raised at a ninety degree angle to the bird. Head to bill measurements were measured (to the nearest 0.01 mm) using Vernier callipers from the bill tip to the centre of the back of the skull (as in Gosler, 1994). Bill length was measured (to the nearest 0.01 mm) using Vernier callipers from the tip of the bill to the bill's base at the skull. The tarsus length used for adults was a measure of the minimum tarsus – from the base of the tarsometatarsus to the notch at the tarsal joint – the measurement was taken using Vernier callipers (to the nearest 0.01 mm) with the foot forming a right angle to the tarsus.

Inter-sexual differences in body mass, skeletal measurements (including tarsus notch length, bill length, head-bill) and feather measurements (including wing length and tail length) were tested for equal variances (F-statistic) before comparisons using independent t-test (Zar, 1974).

2.3.3. Blood Sampling

Blood collection techniques were followed according to the methods used in Ewen (1998) and are detailed in Arden, McLean, Anderson, Maloney & Lambert (1994). Blood samples were taken using venipuncture of the right brachial vein using a 13-mm 27-gauge needle. Blood was collected using heparinised capillary tubes (approximately 70 μl of blood) and stored in Queen's lysis buffer (Seutin, White & Boag, 1991). Pressure was placed on the wound with cotton pads to stop bleeding. Once bleeding had ceased, the individual was released and monitored until recovery was evident. Each blood sample was labelled with the individual's band combination, location and date and stored at -20 C.

Blood sampling of chicks was carried out opportunistically, according to the age of chicks. If a nest was found early in the nestling stage, chicks were sampled between 4-6 days old. However, if nests were found later in the nestling stage (closer to fledging) chicks were sampled as soon as possible with minimum disturbance so as not to cause forced fledging. Blood was obtained from chicks via a small puncture in the medial metatarsal vein with a 13 mm 27-gauge needle. Approximately 30-40 μl of blood was collected and stored using the same methods as for the adult birds. Where nests were found with old chicks and blood sampling equipment was not accessible, feather samples from each chick were taken and stored in plastic bags to be used for sexing analysis.

2.3.4. Nesting biology

During the breeding season (August – February) in 2005 and 2006, individual banded birds were monitored to determine location of territory and breeding partner. Pairs were followed to observe frequent flight paths and breeding behaviours. Females seen with nesting material, flying low (with obvious weight due to egg carrying) or with fast, direct flight were followed to find nest sites. Opportunistic nest searches were also carried out where mating/nesting behaviours were observed.

The characteristics of each nest site were recorded including: tree species, nest lining, nest stage and clutch size. Once nesting was completed or if the nest had failed, each nest was measured with Vernier callipers to determine inner diameter (within the nest rim), outer diameter (including nest rim), inner depth (nest cup) and outer depth (from rim to base materials). Nest cover was determined by placing a digital camera (SONY Cybershot™) horizontally on top of the nest rim and taking a photograph. Each photograph was printed as A4 size and an A4 grid split into 2 mm x 2 mm squares was used to calculate percentage light and dark covering the nest.

Differences between successful (fledged at least one young) and unsuccessful nests in relation to nest cover were tested using the Wilcoxon-Mann-Whitney test (SAS

Institute, 2003). The relationship between percentage of young fledged and percentage nest cover were investigated using Spearman's rank correlations (SAS Institute, 2003).

Apparent nest success is the proportion of clutches laid that result in one or more offspring fledged (Armstrong, Raeburn, Powlesland, Howard, Christensen & Ewen, 2002). To determine the effect of tree species (nest site) on nest success, nests that fledged at least one young were compared to those that failed among high, medium and low preference sites. Preferences were defined as high (the occurrence of more than 10 nests within the tree species), medium (selected more than once but less than 10) and low preference (only single nests found in the tree species).

The average size of nests was calculated by determining the mean inner and outer diameter, inner and outer depth. Nest tree preference and related nest success were tested for differences using Chi-Square goodness-of-fit test. Data regarding the abundance of trees available as nest sites on Tiritiri were not available.

2.3.5. Breeding biology

2.3.5.1. Chick growth

Nests with eggs were monitored every second day to determine hatching date. Chicks from nests with precisely known hatching times were used as points of reference for aging chicks from other nests. Sade (1980) recommended comparing dependable features of morphological change of animals of known age with those of unknown ages, to determine their estimated age. Observations of chicks of known ages were used as reference guides to age chicks (see Figure 2.1).

Figure 2. 1: Photographic guide of Bellbird nestlings of known age up to day 11 of development.

Chicks were measured opportunistically; nestling mass (to the nearest 0.01 g) was measured by electronic scales and head-bill measurements (within 0.01 mm) were taken as per those from adults. However, tarsus measurements from chicks included the full tarsus length (from the base of the tarsometatarsus to above the tarsal joint). A single series of measurements per offspring, from each nest, was taken opportunistically and the age, where not known exactly, was estimated for each chick according to Figure 2.1. Graphs were plotted using Curve Expert 1.3©.

2.3.5.2. Breeding Synchrony

Localised synchrony is relevant to the study of mating strategies since interactions between fertile females and extra pair males are expected to be more frequent between close neighbours (Chuang, Webster & Holmes, 1999). Breeding synchrony is the overlap in fertile periods of females within a population (Stutchbury & Morton, 1995).

The breeding synchrony index (SI) was defined as the average percentage of females fertile on a certain day throughout the breeding season (Björklund & Westman, 1986; Kempenaers, 1993). As in Stutchbury & Morton (1995) and Kempenaers (1993) the fertile period of a female was defined as 5 days prior to the first egg being laid (modal clutch size = 3, plus one day either side for laying delay) until the laying of the penultimate egg . The number of females fertile on each day of the breeding season was calculated as an average percentage of all females within the localised study area (Kempenaers, 1993). Although not all of the females within this study area were found or followed, a significant number of neighbouring territories (22 direct neighbours) within the "Bush 1" area were studied throughout the breeding season. Nests that were from different parts of the island (i.e. not from neighbouring females) were excluded from synchrony index calculations.

2.3.5.3. Nest success

Stage-specific daily probability survival was calculated using Stanley's model (Stanley, 2000). The SAS programme for Stanley's method is available from Ecological Archives (http://www.esapubs.org/archive/ecol/E081/021). As suggested by Armstrong *et al.*, (2002), the starting p-value of the iterative phase was changed from p=0.90 to p=0.99 (as used in Stanley's SAS code) to avoid the programme producing incorrect p value estimates (i.e.: p-value greater than 1). Confidence Intervals were calculated following the Delta method (Seber, 1982) as per Stanley (2000) and Armstrong *et al.*, (2002). The egg laying stage was not included in analysis due to the fact that only one nest was found in the two year study during egg-laying. Therefore, Stanley's programme was edited by deleting calculations for egg-laying (as in Armstrong *et al.*, 2002). Survival rate for the incubation stage (p^1) and nestling stage (p^2) were calculated with the known duration of incubation stage (t^1= 14 days) and nestling stage (t^2 = 16 days), with the following equation:

$$p^{1\wedge t1} \text{ x } p^{2\wedge t2}$$

Stanley (2000) adapted in Armstrong *et al.*, (2002). SAS Version 9© was used to run Stanley's programme.

2.3.6. Primary sex ratios of clutches

Primary sex ratios are an expression of the proportion of males and females produced in a brood. Since there is ambiguity associated with this definition, the term 'sex ratio' from here on indicates the proportion of males in the sample (as in Wilson & Hardy, 2002). Only complete nests were used in analysis of primary sex ratios. These were determined by sexing all of the chicks and unhatched eggs within each clutch.

DNA sampling of nestlings was undertaken as specified in Section 2.3.3. Any unhatched eggs were collected, opened and the remaining embryo stored in 80% Ethanol (EtOH). Blood, tissue and feather samples were used for sexing analysis. DNA sexing methods were adapted from Millar *et al.*,(1992) and details are presented in APPENDIX II.

Primary sex ratios were analysed for deviations from equality using the binomial test (Wilson & Hardy, 2002). Due to the large sample size (n>25), the normal approximation to the binomial test (Zar, 1974) was used. Only broods containing complete nest samples were included in analysis, this included a total of 59 nestlings and 12 eggs from 27 nests. To determine the effect of year and clutch order on primary sex ratio, data were analysed using a Generalized Linear Model (PROC CATMOD, SAS Institute, 2003) (Wilson & Hardy, 2002) using maximum likelihood analysis of variance (approximating the χ^2 statistic).

2.4. Results

2.4.1. Banding and Measurements

A total of 147 Bellbirds (121 males and 26 females) were banded from 2005-2007. Thirty-two birds were banded in 2000 by I. Castro, 11 of which were re-sighted throughout this study. This indicates that the life span of Bellbirds is at least seven years.

Male and female Bellbirds differed significantly in all measurements taken with males consistently larger and heavier than females (Table 2.1). Tail length, wing length and body mass showed the largest differences between the sexes and the distribution of these are presented in Figure 2.2. Male mass ranged from 23-36.5 g, with an average of 29.08 g (±2.1 S.E.). Female mass ranged from 19g-29.25 g with an average mass of 22.46 g (±2.2 S.E.). Male tail length had a mean of 82 mm (± 4 mm S.E.; range: 72-89 mm), while female tail length had a mean of 66.9 mm (±3.4 mm S.E.; range 61-77 mm). The highest degree of overlap between the sexes occurred in tarsus length and bill length. Males had an average tarsus length of 26.42 mm (±0.13 S.E; range 24.8-29.6 mm) and females had an average tarsus length of 25.03 mm (±0.34 S.E.; range 23.18-28.93 mm). Bill length measurements overlapped between the sexes with males having a mean of 14.83 mm (±0.09 S.E.; range 13.07-16.9 mm) and females having an average bill length of 14.05 mm (±0.17 S.E.; range: 12.8-16.16 mm).

Table 2.1: Inter-sexual size differences in adult Bellbirds showing mean (±standard error) for body measurements with the corresponding student's t-values and level of significance.

Sex	**Mass (g)**	**Tarsus length (mm)**	**Bill length (mm)**	**Tail length (mm)**	**Wing length (mm)**	**Head-Bill length (mm)**
Adult ♂	29.08 (±0.19)	26.42 (±0.13)	14.83 (±0.09)	82.02 (±0.54)	85.91 (±0.31)	41 (±0.12)
Adult ♀	22.46 (±0.39)	25.03 (±0.34)	14.05 (±0.17)	66.91 (±0.74)	76.09 (±0.29)	38.07 (±0.26)
t statistic	15.54	3.48	4.17	13.28	23.17	10.96
Significance	**	**	**	**	**	**

** ***P*** value significance <0.001

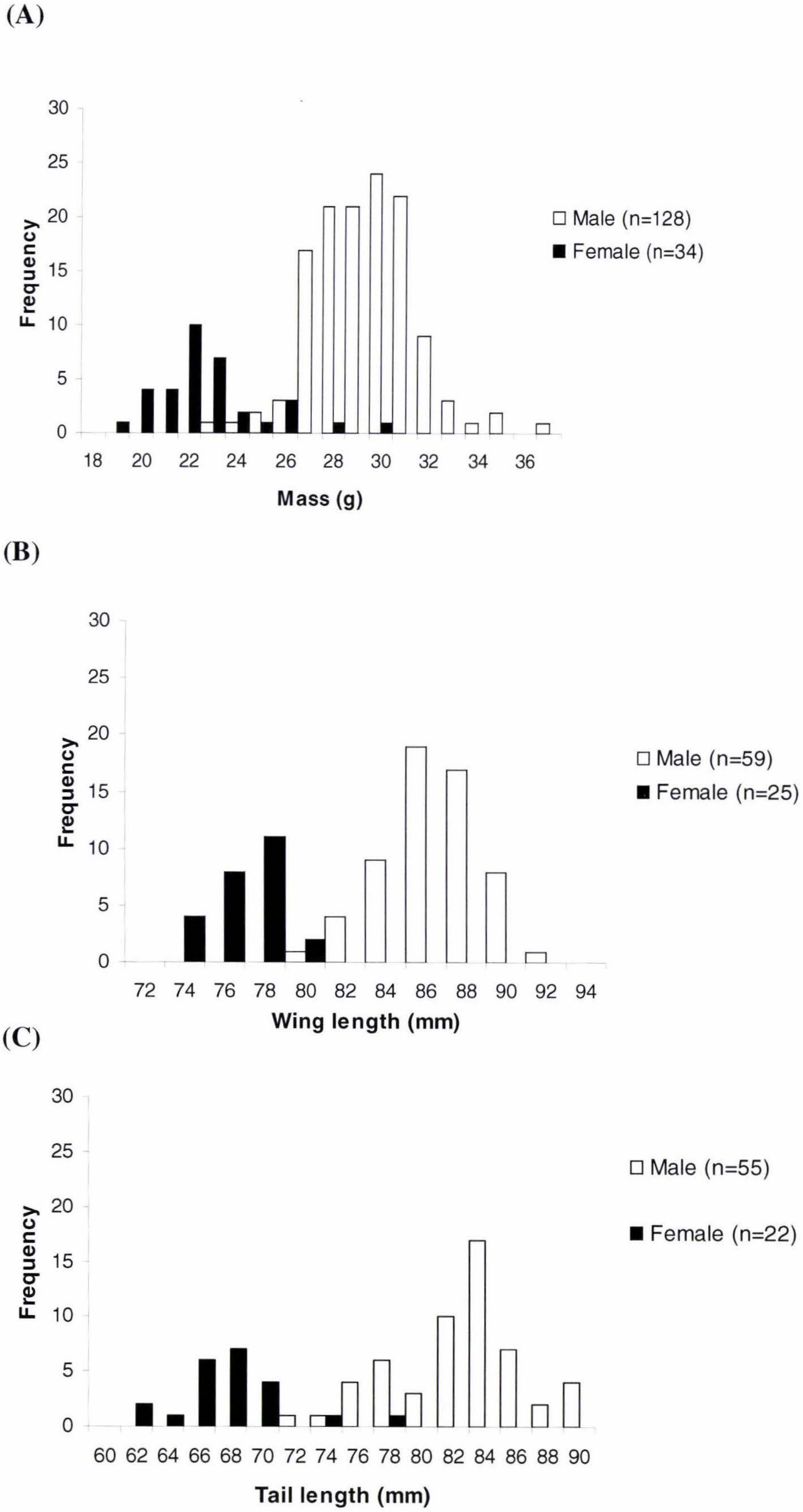


Figure 2.2: Frequency of adult male and female body measurements on Tiritiri showing (A) body mass; (B) wing length; and (C) tail length.

2.4.2. Timing of breeding

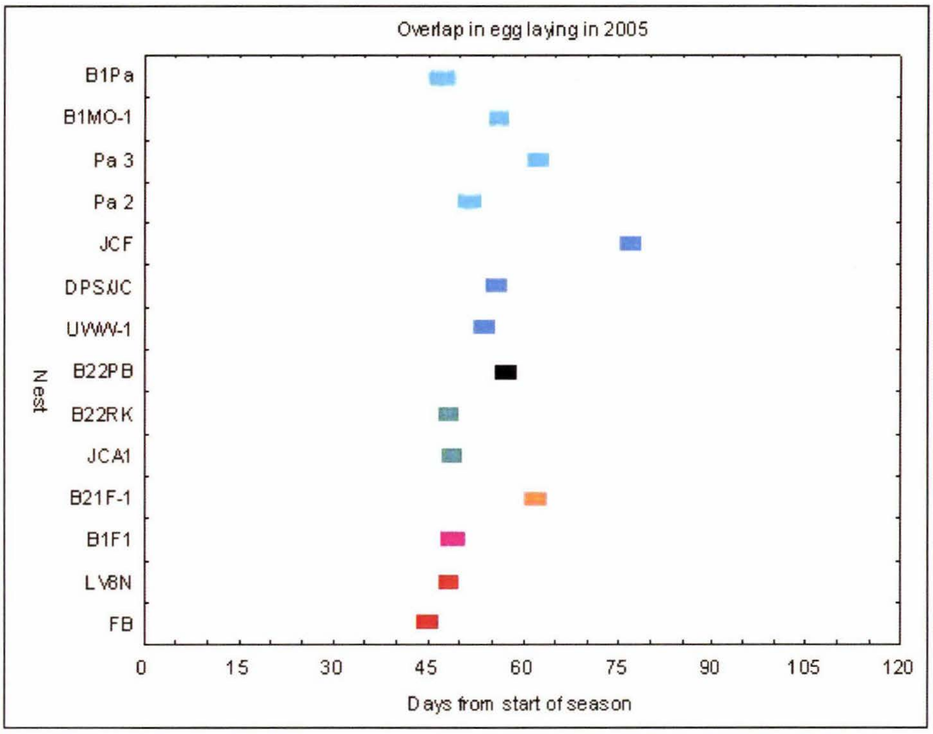
In 2005, the first nest building attempts were observed at the beginning of October. However a high proportion (54%) of nest building occurred from 15th October to 28th October 2005. Incubation occurred from 19th October to 16th November 2005. Nestlings were present from 1st November to 4th December 2005. Eight second clutches were found in 2005 with egg-laying occurring within 17 days of 1st clutch nestlings fledging.

The first 2006 nest was observed with chicks in early September. The majority of nest building (91%) occurred between the 30th October and 30th November 2006. Incubation was observed from 6th November until the 22nd December 2006. Nestlings were present from 22nd September to 8th October 2006 and 19th November to 28th December 2006.

2.4.3. Breeding Synchrony

A total of 19 females in 2005 and 15 females in 2006 were followed through each breeding season. Synchrony of breeding was calculated among neighbouring females within the 'Bush 1' area in 2005 (n= 11) and 2006 (n=14). Bellbirds were found to be reasonably asynchronous in both seasons (see Figure 2.3). Twenty three percent of the target population was synchronous (SI= 23.27%) in 2005 while only ten percent (SI=9.4%) was synchronous in 2006. The median egg laying date for first clutches was on day 58 (28th October; range: day 47-79) in 2005 and day 85 (24th November; range: day 8-103) in 2006 (Figure 2.4).

(A)



(B)

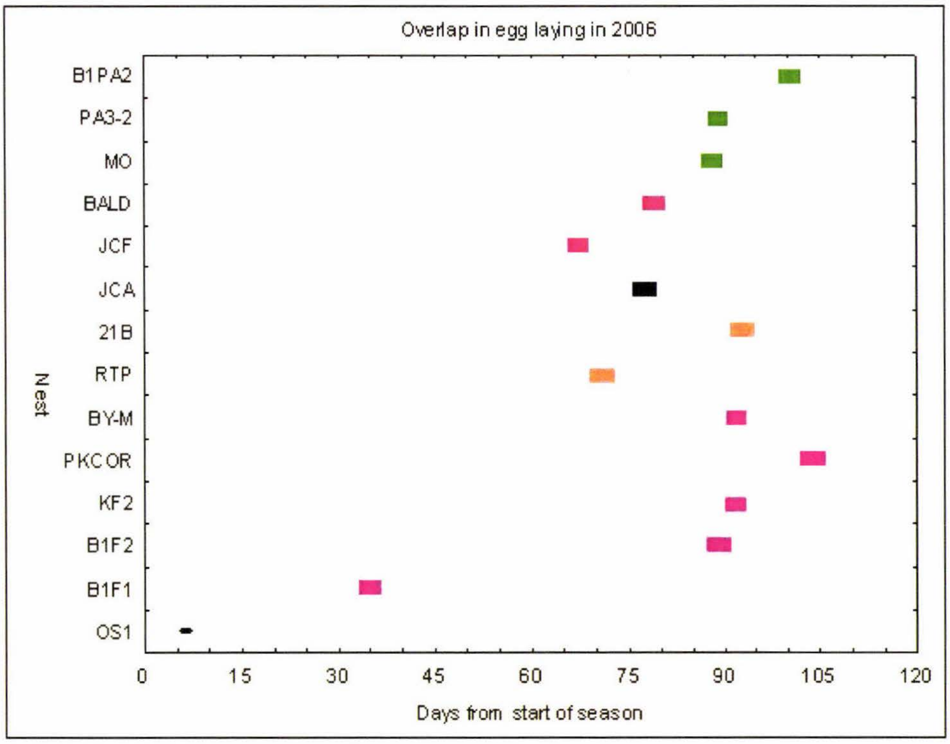


Figure 2.3: Overlap in egg laying dates from the start of the breeding season in 2005(A) and 2006 (B). Colour blocks indicate neighbourhoods where nests are arranged in proximity to each other. Nests OS1, LV8N and FB were located on the opposite end of the island and were not included in synchrony calculations.

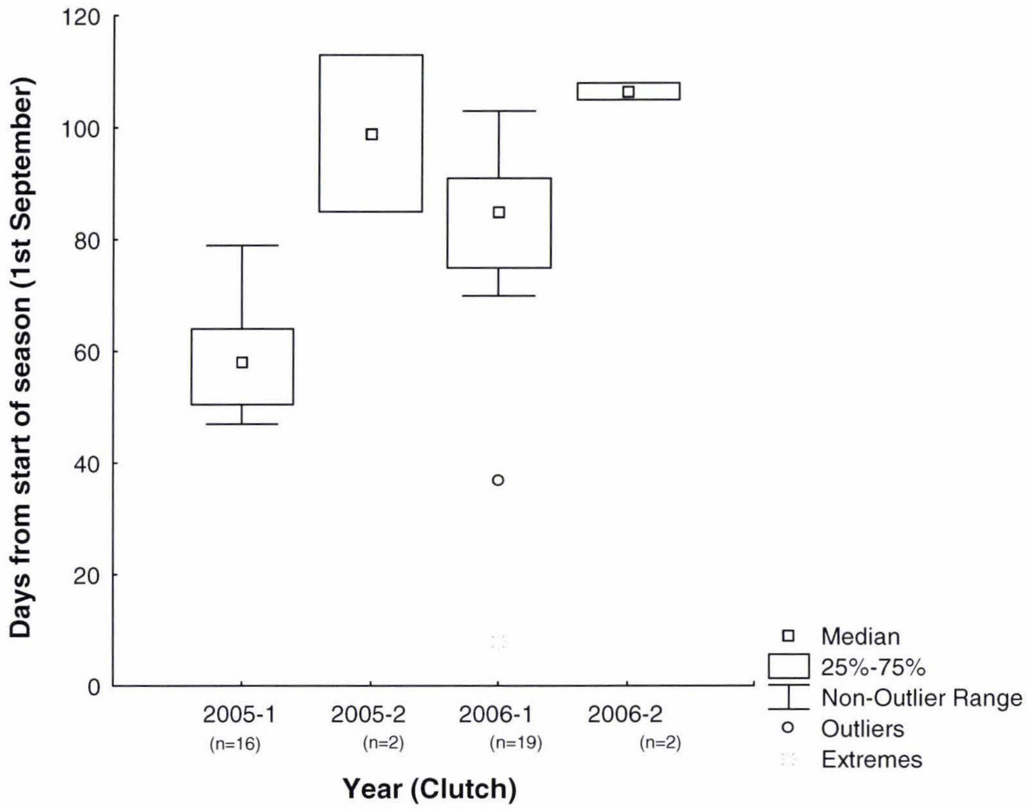


Figure 2.4: Variation in egg laying dates per season. Numbers next to each year represent first or second clutches (1 and 2, respectively) of which the sample sizes are represented in parentheses.

2.4.4. Clutch size, incubation length and nestling dependence

Of the 45 nests monitored, 28 (66%) were 3-egg clutches, 14 (31%) were 2-egg clutches and three (6%) contained 4 eggs. The median clutch size was 3 and the mean (±SE) clutch size was 2.75 ±0.08. Clutch sizes did not differ significantly between 2005 and 2006 (t=0.65, *P*=0.51).

Incubation length was determined from four nests. Median incubation length was 14 days. It was observed to occur twice for 14 days, once for 13 days (with asynchronous hatching occurring) and once for 15 days. However, eggs were also identified as being present in nests once for 17 days and once for 18 days before hatching.

Nestling post-hatching dependence period was observed for 23 nests. Nestlings were seen to remain in nests for a median of 16 days with a range of 15-19 days. Forced fledging occurred at day 10 in four instances, all of which resulted in the survival of at least one chick through continued parental care of the young on the ground. Three of these cases occurred under natural circumstances where nest construction had failed and there was one case of forced fledging due to being handled for sampling. Post fledging dependence was observed as occurring for at least 14 days (n=5).

2.4.4.1. Nestling growth

Logistic regression has been documented as the growth rate for Bellbirds (Anderson & Craig, 2003) as well as many other bird species (Ricklefs, 1967), including the Arabian Babbler *Turdoides squamiceps* (Anava, Kam, Shkolnik & Degen, 2001), White-bellied Swiflets *Collocalia esculenta* and Blue-throated Bee-eater *Merops viridis* (Bryant & Hails, 1983).

The logistic growth curve fitted Bellbird growth patterns reasonably well for mass gain (males: r = 0.90, females: r=0.94) and tarsus growth (males r =0.822, females: r=0.87) (see Figure 2.5). Chick growth showed the fastest increase in all measurements from day 5 to day 8. Due to the high occurrence of forced fledging, nests were not disturbed after nestling age of 12 days. Therefore, data presented represents single measures from individual chicks at specific ages up to a maximum age of 12 days.

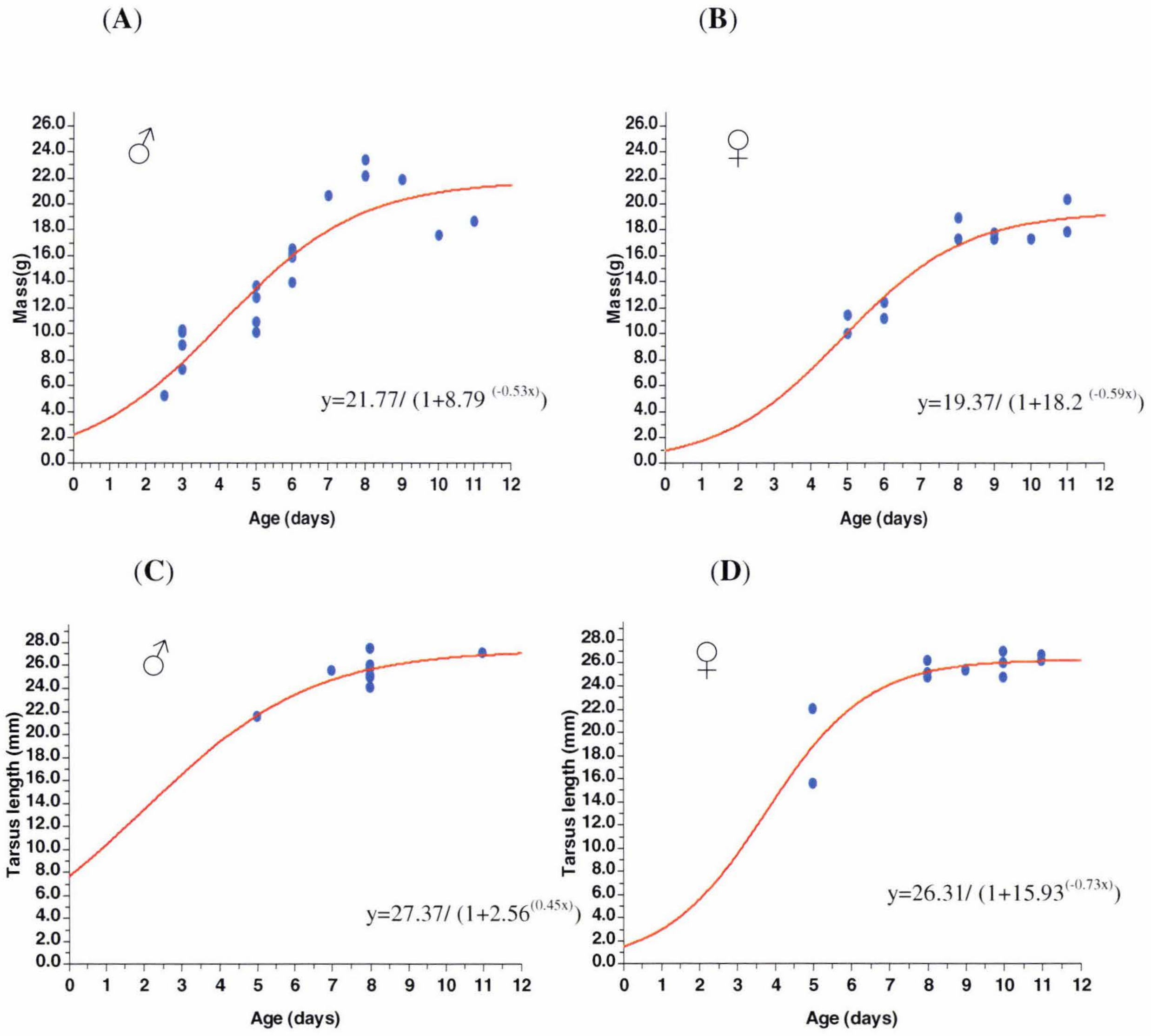


Figure 2.5: Logistic growth curves for Bellbird nestlings. Nestling mass growth over time showing (A) male nestlings (r = 0.90, n=19) and (B) female nestlings (r=0.94, n=11). Nestling tarsus growth over time, showing (C) male nestlings (r=0.822, n=10) and (D) female nestlings (r=0.87, n=12).Growth equations are represented in the lower portion of each graph.

2.4.5. Nesting Biology

2.4.5.1. Nest dimensions and lining

Nests consisted of a stick base built from twigs, roots and grass and an inner cup constructed from finer materials including roots and grass and occasionally fern scales. The cup was heavily lined with an assortment of feathers, most of which included: Kereru (*Hemiphaga novaeseelandiae*), Pukeko (*Porphyrio porphyrio*), Kiwi (*Apteryx owenii*), Kakariki (*Cyanoramphus novaezelandiae*), Morepork (*Ninox novaeseelandiae*), Takahe (*Porphyrio mantelli*), Saddleback (*Philesturnus carunculatus*) and Hihi. Of the 26 nests studied, 14 (54%) were lined with a majority of Kereru feathers, 10 (38%) were lined with majority Pukeko feathers, one was lined almost exclusively with Kiwi feathers and one with Kakariki feathers.

A total of 19 nests were measured. These varied in size: for instance, the mean outer depth (length) of nests was 82.11 mm ± 7.1 S.E. However nests ranged from 38 to 120mm in length, whereas the outer diameters (width) of nests ranged from 43 to 121.1mm, with the mean width of nests 98.49 mm ±4.5 S.E. There was less variation in the inner diameters of nests (mean = 55.7 mm, S.E =1.7, Var =52.7).

2.4.5.2. Nest tree preferences

Nests occurred in a range of tree species with the most frequent being Cabbage tree (*Cordyline australis*). Of 54 nests, 30 (55%) were built in Cabbage Trees (see Figure 2.6) usually under hanging fronds on the side of the trunk. Nests were infrequently built in low preference sites such as semi-cavities within the bark of Pohutukawa (*Meterosideros excelsa*); in entanglements of Wireweed (*Muehlenbeckia spp*), in hanging fronds of tree fern species (e.g.: *Cyathea dealbata*), in Rewarewa (*Knightia* *excelsa*), among Flax (*Phormium tenax*) and in various other species of plants including: Mahoe (*Melicytus ramiflorus*), Karo (*Pittosporum crassifolium*), Manuka (*Leptospermum scoparium*), Broom (*Carmichaelia australis*), Red Matipo (*Myrsine australis*) and Totara (*Podocarpus totara*).

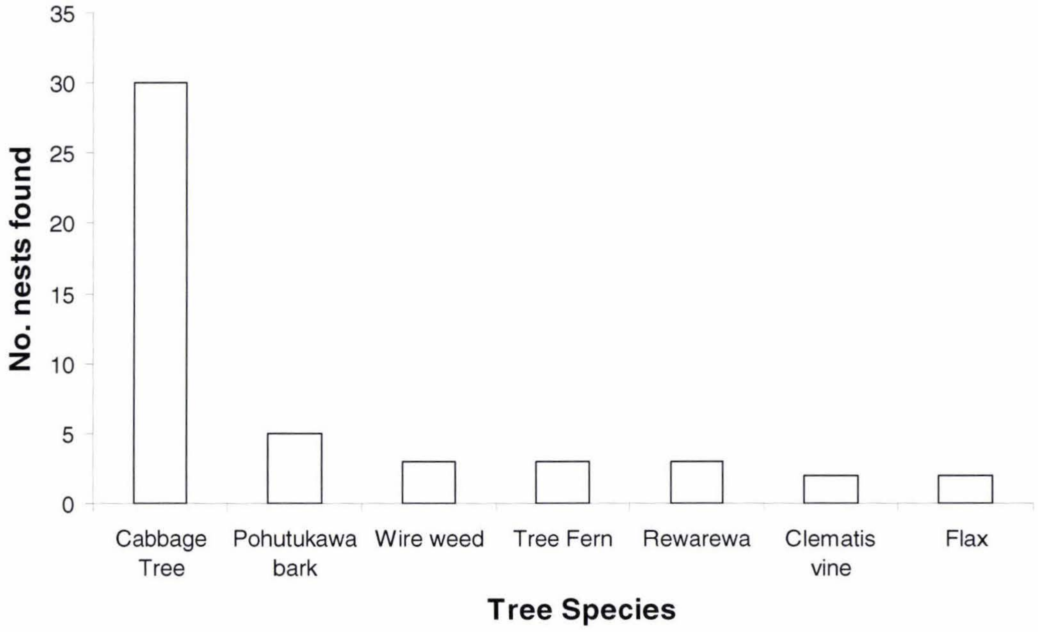


Figure 2.6: Tree species selected for nest sites by Bellbirds on Tiritiri Matangi Island (n=54).

2.4.5.3. Nest tree preference and nest success

There was no selective advantage of nest site between high, medium and low preference tree species. The number of success and unsuccessful nests according to site preference is represented in Figure 2.7. No significant difference was found in the proportion of successful and unsuccessful nests per tree species ($\chi_2=2.84$, $0.25<P>0.1$).

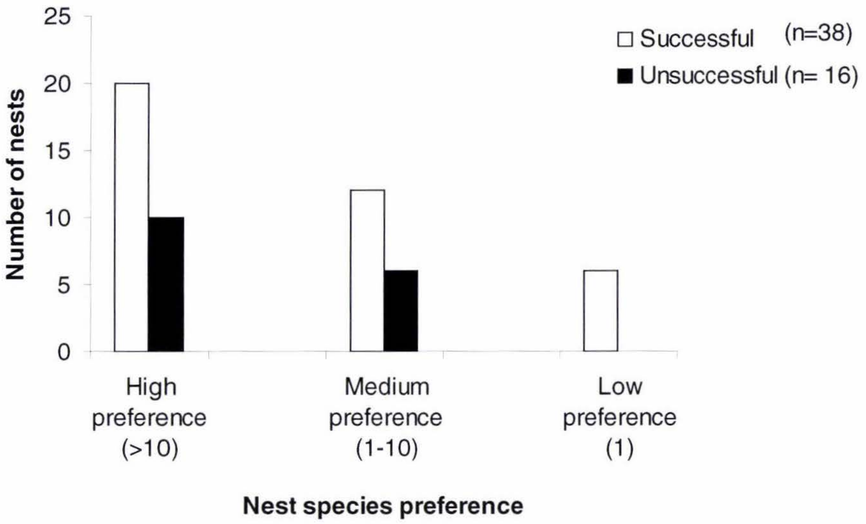


Figure 2.7: Number of successful (fledging at least one young) and unsuccessful nests according to nest species preference.

2.4.5.4. Nest cover

All nests were well concealed and difficult to find, with an average of 92.96% vegetation concealment from above. Nest cover ranged from 72% in the most exposed nest to 100% in the most concealed nests. No significant difference in the percentage cover over the nest was found between nests that fledged at least one young and those that did not fledged any young (Wilcoxon normal approximation: Z=-0.46, df=1, *P*=0.646). There was also no correlation between the percentage of young fledged and nest concealment (r_s=-0.003, n=34, *P*=0.98). Examples of nest cover are shown in Figure 2.8.

Figure 2.8: Examples of nest cover over Bellbird nests on Tiritiri in 2005 and 2006. Percentages light and dark were calculated for each photo representing nest cover.

2.4.5.5. Nest Success

The mean number of nests per female was 1.55 ± 0.19 S.E in 2005 (n=20) and 1.25 ± 0.11 S.E in 2006 (n=16). The average number of chicks raised per nesting attempt per female was 1.5 ±0.24 S.E in 2005 (n=20) and 1.4 ± 0.29 S.E in 2006 (n=16). For comparison, three methods of calculating nest success (Apparent, Mayfield and Stanley's) were used in this study and are presented in Table 2.2. Of 23 nests followed in 2005, 15 produced at least one fledgling giving an apparent nest success of 65%. In 2006, of 20 nests followed, 11 produced at least one fledgling indicating apparent success of 55%. Similar values were found for nest success using Mayfield's and Stanley's methods. However, due to the increased accuracy in determining nest success using Stanley's method (Armstrong *et al.*, 2002); values of nest success are deduced from this method and presented hereafter. The nestling stage had a consistently higher probability of survival (78% in 2005 and 71% in 2006) than the incubation stage (57% in 2005 and 58% in 2006). Estimated overall nest success (and 95% confidence limits) from egg-laying to fledging was 45% (14-79) for 2005 and 41% (18-69) for 2006. Nest success of Bellbirds on Tiritiri has not changed in 27 years, since it was estimated as 44% in 1979 using Stanley's method (Anderson & Craig, 2003). Nest failure usually occurred after storm events. However there was one documented case of infanticide (killing of young) by Tui and one suspected case of egg removal by Indian Mynah (*Acridotheres tristis*).

Table 2.2: Estimates of nest success of Bellbird breeding on Tiritiri using Apparent, Mayfield and Stanley's estimates.

	2005 Nest Success (%) (n=55)			**2006 Nest Success (%) (n=57)**		
Nest Success Method	Incubation stage	Nestling stage	Overall (95% CI)	Incubation stage	Nestling stage	Overall (95% CI)
Apparent success	69	78.9	**65**	64	92	**55**
Mayfield estimate	55	74	**41**	57	83	**48**
Stanley's estimate	57	78	**45** **(14-79)**	58	71	**41** **(18-69)**

2.4.6. Primary Sex Ratios

Seventy-one chicks and embryos from 2005 and 2006 were analysed for sexing. Of these, only three samples were not able to be amplified to produce a result. Twenty-nine chicks and six embryos were sexed from 14 nests in the 2005 season as well as 27 chicks and two embryos from 12 nests in 2006. Analysis of sex ratios indicated no significant deviation from the binomial distribution in either year. There were proportionally more males (65.7%) than females (34.3%) produced in 2005, although this is not statistically significant ($0.05<p<0.1$, n= 35). There were equal numbers of males (51.8%) and females (48.2%) produced in 2006 ($p>0.5$, n= 27).There were no significant effects of clutch number (1st clutch: χ^2= 0.89, df= 1, P= 0.208, n= 57; 2nd clutch: χ^2= 0.14, df= 1, P= 0.7110, n= 5) or year (2005: χ^2= 0.17, df= 1, P= 0.667, n= 36; 2006: χ^2= 0.36, df= 1, P= 0.639, n= 26) on sex ratio in the generalised linear model.

2.5. Discussion

2.5.1. Adult Measurements

Bellbirds on Tiritiri exhibit a large sexual size dimorphism as well as sexual plumage dimorphism (dichromatism). As reviewed by (Owens & Hartley, 1997), large size dimorphism is associated with polygamous mating systems and large differences in brood provisioning. Also, large sexual plumage dichromatism (especially in iridescent blues, purples and blacks) is associated with the occurrence of extra-pair offspring (Owens & Hartley, 1997). The differences in levels of brood provisioning by each sex are presented in Chapter Three and the occurrence of extra-pair offspring is presented in Chapter Four.

2.5.2. Nesting and Breeding Biology

The nest site characteristics described in this study were similar to those reported by Anderson & Craig (2003) and Oron (2002). However, this research revealed a distinct preference for Cabbage Trees as nest sites which differs from the results in 1979 when Bellbirds nested primarily in the hanging skirts of Tree Ferns (Anderson & Craig, 2003). One reason for this difference in preference may be due to changes in the vegetation on Tiritiri, which has altered dramatically since 1979 when the island was primarily grassland and scrub (Esler, 1978). Cabbage Trees are not the predominant vegetation on Tiritiri (pers.obs) and therefore Bellbirds currently disproportionately choose this species for nesting sites. Yet the selective advantage of this choice is unknown, since nest success did not differ according to tree species. Cabbage Trees have straight trunks with rounded heads thick with foliage that droop when the tree is older and have characteristic dead hanging fronds when younger

(Salmon, 1980). Nests may have preferentially been built in Cabbage Trees due to the physical aspects such as dense cover of vegetation. It may be possible that due to their height and physical isolation from the bush, nest detection was easier in Cabbage trees. However, nest searches involved considerable time and effort in finding all possible nests in all possible locations, by carefully searching all trees within Bellbird nesting territories. Therefore, it is unlikely that these results were due to biases in nest detection.

Percentage cover represents the vegetation concealment above the nest. Nests with 100% cover can almost completely conceal nestlings (from above and below) excluding the side that the parents enter to feed and as a result could be viewed as a semi-cavity. Cavity nests have a myriad of benefits relating to protection from predation for the incubating female and the eggs/young. However, along with these benefits some disadvantages occur. For instance, parasite loads in cavity nests far exceed that of open nests and can have detrimental effects on the growth and survivorship of offspring (Alves, 1997; O'Brien, Morrison & Johnson, 2001). A semi-cavity nest can provide almost complete cover, protecting the nest from predation, however it is also still open to the elements (i.e. sunlight, wind etc) that could influence nest microclimate thereby decreasing the levels of ectoparasites (Heeb, 2000).

Nest success for Bellbirds in this study (45% in 2005 and 41% in 2006) was lower than that of cavity nesting species on Tiritiri (e.g. Red-crowned Kakariki, 60.5%, Ortiz-Catedral, 2006) and similar to other open nesting passerine on the island (e.g.

North Island Robin, *Petroica australis*, 41%; Armstrong *et al., 2002*), all of which were calculated using Stanley's method.

Considering the high concealment of nests as well as the increased habitat available for nesting since 1979, the current nest success should be higher than that of past studies. It is interesting to note that Bellbird nest success has not increased since 1979, despite the eradication of Kiore in 1993 (Graham & Veitch, 2002). Both studies used Stanley's estimate of nest survival and were therefore comparable, however, sample size was low (n=17) for the study by Anderson & Craig (2003) in 1979 compared to the sample sizes in this study (n=55 in 2005 and n=57 in 2006). Therefore the two studies are not directly comparable; however a trend does exist for similar levels of nest success in this population over time. It could be speculated that nest success increased in the period between the two studies after Kiore eradication, and is currently restricted due to resource limitation at high population density (Lack, 1968).

Numbers of avian predators may have increased over time along with Bellbird numbers. However, evidence of nest predation in this study was limited to one case of predation competition by Tui and one suspected case of egg removal by Indian Mynah (*Acridotheres tristis*).

The most common cause of nest failure appeared to be storm damage. When nest abandonment occurred, it was at the egg stage and usually after a storm event. In contrast to Anderson & Craig (2003), who reported consistent abandonment of single chick nests, there were no cases of nestling abandonment recorded in this study. In fact, this research recorded four nests successfully raising single offspring, and on three occasions successfully raising a single chick on the ground after the nest had fallen.

The average clutch size of Bellbirds nesting on Tiritiri in 2005 and 2006 was slightly smaller than in 1979 (from $\bar{x}$=3.6 to $\bar{x}$=2.75). Clutch size evolves within the balance of food availability, parental ability for feeding young, as well as optimisation of reproductive output for female birds (Lack, 1968). The size of a breeding population can also act as an influence of clutch size evolution, with clutch sizes varying at differing population densities due to the pressures on food resources (Lack, 1968). The Bellbird population on Tiritiri has increased from estimates of 150 individuals in 1977 (Anderson & Craig, 2003) to conservative estimates of over 500 individuals (OSNZ census data suggests at least a three-fold increase since 1989; Veitch *et al*, *pers.comm*). The life history traits of territorial species can be understood through the ideal despotic distribution (Goss-Custard & Sutherland, 1997), as a population increases territory, holders either hold reduced or sub-optimum territories, either of which reduce reproductive output (Goss-Custard & Sutherland, 1997). If territory holders are affected by population density, as predicted by the ideal despotic distribution, there should be subsequent changes in reproductive output and success over time. This can be seen in the reduced average clutch size in Bellbirds since 1979. However, sample sizes from the 1979 study by Anderson & Craig (2003) were considerably smaller than this study and although a trend exists, the two studies cannot be directly compared.

Asynchronous breeding was observed in this population of Bellbirds. This has implications for paternity and the possibility for polygamy, since asynchronous breeding increases the amount of time that fertile females are available to engage in extra pair copulation, which could also result in promoting polygamy (Emlen & Oring, 1977).Therefore, due to the level of asynchrony in this population, relatively high levels of extra pair paternity are expected (see Chapter 4). However, sperm storage duration is an important part of the female's fertility period and an early copulation could potentially fertilise one or more eggs (Birkhead & Moller, 1992). If female Bellbirds are able to store sperm, the assumed 5 day fertility window could be greatly increased. Since all females would have this ability, the proportion of overlap in breeding within the population should not change. If early extra-pair copulations at temporal distance from egg laying can be successful, it would also affect the reproductive output of cuckolded males within a pair. Considering the high level of asynchrony in this population, relatively high levels of extra pair paternity are expected.

New Zealand passerines are characterised by having longer incubation stages compared to their Australian counterparts (Franklin & Wilson, 2003), except for the Meliphagidae which have consistently short incubation and nestling periods throughout their range (Franklin & Wilson, 2003). The incubation period of Bellbirds has been reported as lasting 14 days (Anderson & Craig, 2003; Heather & Robertson, 1996). The median length of time incubation occurred during this study was 14 days; however it ranged from 13 days to 15 days. This is consistent with incubation periods for the other New Zealand honeyeater (Tui: c.14 days; Heather & Robertson, 1996) and for the Australian honeyeaters (Regent honeyeater: c.14 days, Geering & French, 1998; Crescent honeyeater: c.13.5days, Clarke & Clarke, 2000). However, differences in nestling periods were observed in this study. Nestlings remained in the nest for an average of 16 days, compared to an average of 14 days in 1979 (Anderson & Craig, 2003). This effect may be due to the reduced nest predation pressure since the removal of Kiore.

Chick growth followed a typical logistic growth rate (Ricklefs, 1967) similar to that shown by a large number of bird species (for examples see Anava *et al.*, 2001 and Bryant & Hails, 1983). The method of chick comparison to those of known ages resulted in accurate estimation of age, shown by the fit to the logistic regression. The low sample sizes and small range of sample dates potentially influence the specific fit of the logistic regression to the data, especially since there was limited ability to predict the start of growth (i.e. Day 1). However, the logistic regression fitted the growth pattern of Bellbirds in the study by Anderson & Craig (2003), which used chicks of known ages. Larger samples taken at early stages of the nesting cycle would increase the accuracy of the results observed. The rate of attendance and quality of food provided to the nestlings by their parents were not analysed as a factor potentially influencing growth, due to methods of data collection and limited time frame available.

2.5.3. Primary Sex Ratios

Since Trivers & Willard (1973) put forward the argument that females adaptively allocate the sex of their offspring, there has been an enormous amount of attention on sex ratio studies. The development of molecular techniques for sexing birds (Griffiths, Daan & Dijkstra, 1996) has increased the attention of scientists aiming to test this hypothesis. There is evidence that females can produce skewed sex ratios (per clutch) dependent on their condition (Whittingham & Dunn, 2000), age (Blank & Nolan, 1983), environmental variability (Sheldon, 1998) and attractiveness of their mate (Ellegren, Gustafsson & Sheldon, 1996). However, many studies show inconsistent results and weak or no evidence when testing the influence of the same variables (e.g.: maternal condition: Whittingham, Valkenaar, Poirier& Dunn, 2002; environmental variability: Budden & Beissinger, 2004; and male attractiveness: Rindstaff, Buerkle, Casto, Nolan & Ketterson, 2001). There have also been suggestions of publication biases in terms of positive results (Ewen, Cassey & Moller, 2004) and recent meta-analysis of all molecular sexing techniques and statistical studies of sex ratios found no pattern of facultative sex ratio control in birds, beyond that which could be explained by sampling error (Ewen *et al.*, 2004).

The sampling of a complete clutch for primary sex ratio analysis is challenging since early mortality of eggs and dump laying are very difficult to detect (Ewen *et al.*, 2004) and see (Budden & Beissinger, 2004), possibly having an effect on the outcome of sex ratio analysis (Ewen *et al.*, 2004). To counteract this complication, close observation of nests and early sampling of chicks/unhatched eggs needs to be conducted. Also, only those studies explicitly having analysed all offspring (including unhatched eggs) should be included in meta-analyses and comparisons. This study only included complete nests, from which each egg and or chick was sexed, when determining the sex ratio. In this instance no significant difference in the proportion of male and female offspring was detected.

Molecular sexing was used to analyse the primary sex ratio in this study. These techniques require the identification of specific loci in males and females. The W (female) chromosome is smaller and contains more 'junk' or 'nonsense' DNA (Stefos & Arrighi, 1971). In samples containing small concentrations or poor quality DNA the W chromosome may not amplify causing allelic dropout (Dr. Leon Huynen, pers.comm.). Considering the high proportion of males in the 2005 samples, it is possible that the DNA had degraded in storage causing a higher percentage of allelic dropout and therefore creating a seemingly higher proportion of males. Future research should analyse the DNA samples as soon as possible, within a year of collection, to avoid this potential issue.

2.5.4. Recommendations for future management

Of 179 birds banded since 2000, six individuals suffered injuries from the bands. All injuries were sustained post-banding and involved colour split and colour wrap bands. Four males were affected by injury due to bands, all involving the back toe (hallux) and claw becoming caught and immobilised in the colour band. Due to the behavioural nature of honeyeaters feeding on nectar in the forest, Bellbirds rotate and hang upturned to access flowers (pers.obs). The physiological aspect of the anisodactyl foot (with three toes forward and one toe backward (McGowan, 2004) as well as the rotation to access nectar leads to an expectation of some impact of banding on honeyeaters. However, the nature of the injuries to females differed from that of males; no females were found with the hallux caught in a band. Two females sustained injuries to the foot; both lost all use of the foot while the band remained on. One female was found emaciated with lesions and an abscess on her foot due to a colour split band slipping down over the foot which resulted in the loss of a toe. Females sustained worse injuries than males in all cases. The New Zealand banding office was contacted with the information and permission was granted to band female Bellbirds on Tiritiri with "B" sized bands.

2.6. Conclusion

Bellbirds exhibit relatively low breeding success considering the maturation of the forest environment since the previous study. Factors contributing to the low success should be further investigated. Population density could be constraining success despite the removal of mammalian nest predators and increased vegetation on the island. Breeding was relatively asynchronous in 2005 and extremely asynchronous in 2006. Incubation periods were relatively short which is consistent within the Meliphagidae family. Nestlings were dependent for longer periods of time than presented in past studies, possibly due to environmental changes. Nest site selection was for well concealed sites, particularly in Cabbage Trees. No statistical biases in primary sex ratios were found.

CHAPTER THREE: Social Breeding Parameters

Female Bellbird on nest.
Photo: Taneal Cope, 2006.

3.1. Abstract

Bellbirds appear to exhibit intense intrasexual competition within males and females and as a result have complex social systems. Investment in offspring is not limited to gamete production in this species, but consists of high levels of biparental care that were not associated with brood size or sex ratio. However, investment in offspring is not equal between the sexes with females attending the nest more frequently, for longer and with shorter intervals between visits than males. Reduced parental investment by males is often associated with polygamous mating systems. In support of this argument, extra territorial forays were common and extra pair males were frequently observed visiting the nests of other pairs. In addition, extra pair copulations were witnessed and one confirmed case of polygyny and polyandry occurring that the same nest (polygynandry) was documented. However, social monogamy was witnessed in the majority of social bonds and high fidelity to mates and nest sites occurred between clutches and between years. The results obtained from this study present the first evidence that the social bonds in Bellbirds may not reflect their true mating system.

3.2. Introduction

The term 'mating system' refers to the general strategy employed in obtaining mates and can include the number of mates acquired, the manner of mate acquisition, the presence or characteristics of pair bonds and the patterns of parental care provided by each sex (Emlen & Oring, 1977). Social mating systems are generally defined according to the nature of the pair bond between mated individuals (Lack, 1968). Social monogamy is the apparent exclusive association of one female and one male over a defined period of time and in birds is closely related to parental care (Ligon, 1999). If a male and a female stay together to raise one brood, successive broods or for successive years, the social mating system is termed monogamy (Lack, 1968). In view of this definition, the majority of bird species (~90%) are considered monogamous (Lack, 1968).

Conflict of interest between the sexes

Parental investment is any investment in offspring that increases the offspring's chance of survival at the cost of reducing future reproductive value of the parent (Trivers, 1972). Forms of parental care include production of gametes, preparation of nests, care of eggs (incubation and guarding) and provisioning of young (nestlings and fledglings) (Clutton-Brock, 1991). Due to the differential investment by each sex in gametes, the sex that invests more (usually the female) becomes a limited resource for the other sex (males) (Trivers, 1972). This differential investment is what drives intrasexual competition, usually manifested as competition between males for access to females. Females in monogamous species often perform most of the parental care and hence suffer higher mortality (Trivers, 1972). This differential mortality increases the male bias in the population (Ligon, 1999). Theoretically, if the sex ratio of a population is equal then an individual will maximise the number of offspring produced through monogamy and biparental care (both parents care for the young), as opposed to male desertion leaving the female to raise the clutch alone (Lack, 1968). This requirement for biparental care to ensure survival of offspring is the most likely cause for the evolution of monogamy (Ligon, 1999).

The cost of producing gametes and raising offspring reduces the parents' survival or future breeding success (Clutton-Brock, 1991). The production of eggs is a substantial cost accruing to approximately thirty percent of the daily basal metabolic rate (Robbins, 1983 in Clutton-Brock, 1991). Active incubation can require between ten and thirty percent of the basal metabolic rate in passerines (King, P.E., 1973 in Krebs & Davies, 1991). Notwithstanding this energetic cost, the time taken to incubate eggs is time allocated away from other activities (e.g.: feeding) that could increase the individuals' fitness. Feeding young and juveniles has been shown to be substantially costly (Clutton-Brock, 1991) and consists of time that could be allocated to other activities (Scott, 1980).

As individuals act to maximise their own reproductive success, a conflict between the sexes transpires. Each parent would benefit from the other individual investing relatively more in the current reproductive success so as to reduce the need for intense investment by themselves (Trivers, 1972). This conflict of interest results in various tactics employed by each sex for increasing individual reproductive success. Trivers (1972) suggested that all males should engage in mixed mating strategies to increase their reproductive success by investing in their current brood in addition to seeking fertilisations with females outside of the pair bond. Female mixed reproductive strategies are reliant on a similar equilibrium, the female needs to balance cooperation by her mate, in terms of parental care, yet seek benefits from extra-pair matings (Moller, 2000); including genetic benefits (Birkhead & Moller, 1992), material benefits through increased parental care or nest defence (Davies, 1986) or fertilisation benefits (Sheldon, 1994). In summary, individuals are expected to maximise their own fitness through exploiting the care of the other parent (Houston, Székely & McNamara,

2005) or by taking advantage of the environmental potential for polygamy (Emlen & Oring, 1977).

Parental Care

In biparental species, male parental investment can include: mate provisioning; territory and resource defence; nest, brood and female defence; and brood provisioning (Trivers, 1972). However, due to internal fertilisation, uncertainty over paternity is expected to increase the variation in male reproductive success and hence parental care compared to females (Trivers, 1972). If the level of paternity varies then parental care should also vary due to the trade-off between current and future reproductive success (Williams, 1966; Westneat & Sargent, 1996). Males should not invest heavily in current reproductive success if paternity is uncertain since parental care can be costly.

Parental care is expected to vary based on environmental conditions or parent condition (Carlisle, 1982; Clutton-Brock, 1991); brood need including brood size (Carey, 1990) and sex ratio (Stamps, 1990); paternity (Moller and Birkhead, 1993); tradeoffs between current and future reproductive success (Williams, 1966; Sargent & Gross, 1985) and the investment of the other parent (Johnstone & Hinde, 2006). Predation is also a factor to be considered in the behaviour of adult birds surrounding a nest (e.g.: Conway & Martin, 2000). Movements to and from a nest should be inconspicuous as not to attract attention of possible predators. In light of this argument for Bellbird behaviour and considering the highly inconspicuous nature of Bellbird nests (see Chapter two), adult nest attendance may be associated with nest exposure.

Territoriality and social behaviours

The defence of resources occurs according to the costs and benefits to the defender (Pyke *et al.*, 1996). If exclusive access to a resource (for survival or reproduction) results in increased fitness to the defender, a disparity between territory and non-territory holding individuals evolves (Pyke *et al.*, 1996). Territorial behaviour is defined as aggressive behaviour by an individual that occurs within a certain location toward certain individuals, eliciting submissive responses, in a repeated manner (Pyke *et al.*, 1996). Territory defence can include a range of behaviours, such as advertisement of presence through song and chasing intruders from a territory, usually determined by the behaviour of the intruder (Peek, 1972). Territorial behaviour is common in nectivorous birds, for example Hummingbirds (Gass, Angehr & Centa, 1976), Sunbirds (Gill & Wolf, 1975) and the Meliphagidae honeyeaters (Pyke *et al.*, 1996) and usually increases with increased quality of resource (Powers, 1987).

A large function of territoriality also involves the defence of breeding resources including access to females (paternity guarding). Active defence of a female during the fertile period helps to ensure paternity of offspring (Birkhead & Moller, 1992). Experiments that removed male territory holders resulted in frequent observations of extra-pair copulations involving his female (e.g.: Western Bluebirds, *Sialia mexicana*, Dickinson, 2003).

The defence of resources within a territory can occur through song, visual displays and chasing (Peek, 1972). The essential role of song in territory defence has been demonstrated through experimental muting or removal of territory males. Red-winged Blackbirds that were experimentally muted had less success preventing territorial intrusions (Peek, 1972). In addition, removal of Great Tit (*Parus major*) territory holding males resulted in replacement by a new male within a few hours, unless the territory males' song was used as a playback within the territory which then resulted in the territory remaining vacant for a longer period of time (Krebs, 1971). Extra-territorial forays and intrusions are often associated with seeking extra-pair copulations, in both males (e.g.: Reed Buntings, *Emberiza schoeniclus,* Marthinsen, Kleven, Brenna & Lifjeld, 2005; Splendid Fairy-wren, *Malurus splendens,* Rowley & Russell, 1990) and females (e.g.: Yellow-breasted Chat, *Icteria virens,* Mays & Ritchison, 2004).

The spatial and temporal distribution of essential resources (e.g.: nectar) can influence the rate of social interactions (Emlen & Oring, 1977). However, all social interactions, including song and direct physical encounters, are likely to occur at a higher rate with increasing density. Population density and the availability of territories may influence the numbers of individuals present in the population that do not have a breeding territory, referred to as "floaters" (Arcese, 1989; Ewen, Armstrong & Lambert, 1999). Studies on populations including "floater" males indicated that these males were relatively successful at obtaining extra-pair fertilisations (Ewen *et al.*, 1999). Hence the increased selection for breeding territory defence in males.

Based on the argument by Trivers (1972) that females are a limited resource for males, competition between males for access to females should be intense. Male competition can take the form of direct encounters, which can lead to dominance hierarchies in a population (Emlen & Oring, 1977). Aggregations of males that are initiated by a dominant male can occur to signal individual quality in context of other individuals or it may act as a pooling courtship and advertising strategy to attract females to the enhanced stimulus (Emlen & Oring, 1977). Male aggregations with subsequent female attraction to the area have been observed in many of the Meliphagidae species (Pyke & O'Connor, 1989).

In situations where males provide parental care, males may become a limited resource for females (Davies, Hatchwell, Robson & Burke, 1992). Female competition for mates (either for male parental care or for access to territorial males) is usually visible as female-female aggression and has been shown in starlings, *Sturnus vulgaris,* (Sandell & Smith, 1996) and Red-winged Blackbirds, *Agelaius phoeniceus*, (Yasukawa & Searcy, 1982). Recent studies have shown that female song has been underestimated in passerines, with a large number of tropical bird species exhibiting female territorial song (Slater & Mann, 2004).

The scope of this study

Bellbird social behaviour and parental care provides an interesting framework for understanding social interactions and parental care in birds.

Bellbirds are documented as socially monogamous (Anderson & Craig, 2003) yet they have female biased biparental care (Anderson & Craig, 2003). Inequality in parental investment (usually manifested as lower male parental care) predisposes a species to respond to changes in the environmental potential for polygamy and to become opportunistically polygynous (Emlen & Oring, 1977). This is supported by the finding that high levels of extra-pair fertilisations tend to occur when males play a relatively insignificant role in raising the young (Moller & Cuervo, 2000).

However, species that show greater sex role convergence (e.g.: both sing and defend resources) are expected to have lower levels of extra-pair fertilisations (Slater & Mann, 2004). Bellbird males and females are fiercely territorial (Craig & Douglas, 1986) and both sing complex songs (Brunton & Li, 2006). Females are particularly aggressive to other females (Brunton *et al.*, *in review*). Female-female aggression could be considered as a way of reducing male polygyny, defending male parental care and nesting resources, or preventing intra-specific brood parasitism (Sandell & Smith, 1997).

The main aim of this chapter is to describe the social behaviours of Bellbirds including parental investment and territory defence. Other aims include documenting the social mating system of Bellbirds, in the context of monogamy (exclusive access of one male to one female for breeding), polygyny (one male with breeding access to more than one female) and polyandry (one female with breeding access to more than one male). The role of female song in Bellbird social behaviour is also considered in the context of the current hypotheses for female song in other species studied.

3.2.1. Objectives

1. Describe the parental investments of male and female Bellbirds throughout the breeding cycle.
2. Document differences between male and female parental investment.
3. Describe the social interactions of Bellbirds within breeding territories during nesting.
4. Determine relationships between individual territory defence, territory characteristics and intrusions.

3.3. Methodology

3.3.1. Nest observations

A total of 18 nests were followed over the 2005 and 2006 nest seasons and 13 females and 14 males were observed over this time. Each Bellbird within the nesting territory was identified by colour bands. Birds were caught, banded and monitored as per Chapter two.

Nests were observed continuously for one hour periods at a distance where observations were not impaired but presence of the observer did not appear to disturb the birds. The majority of nests (13/18) were observed once, however, five nests were observed twice throughout the nesting period. At each nest the time of day, weather conditions, number and age of chicks as well as any additional observations were recorded. Behaviours recorded involved those directed towards nesting as well as social interactions and are outlined and defined in Table 3.1. Behaviours were timed with a stopwatch and recorded to the nearest second. Frequency of visits (number of visits per hour), length of visit and length between visits were used as the main components of parental attentiveness were used as the main components of parental attentiveness for this study.

Nest stages were defined as incubation, chick (young and older) and fledgling. Data for the chick stage were divided into two levels (as in Clarke & Clarke, 1999); early (days 1-7) and late (days 8-16) levels of development.

Table 3.1: Definitions of documented behaviours during Bellbird nest watches.

Behaviour	*Definition*	*Reference*
NESTING		
Arrival	*The exact time the adult bird was on the nest.*	Clarke & Clarke, 1999
Departure	*The exact time the adult bird left the nest.*	Clarke & Clarke, 1999
Approaching	*Adult bird was within 5m of nest, or directed movement toward nest within 10m.*	Authors' definition
TERRITORY DEFENCE		
"Short song"	*A single syllable repeated short song (generally < 3 seconds)*	Authors' definition
"Long song"	*A complex song including more than one syllable (generally >3 seconds)*	Authors' definition
"Chase"	*A directed aggressive movement toward another bird*	Authors' definition
"Territory encroachment"	*Any bird found within 10m of the nest, that was not part of the breeding pair or offspring*	Authors' definition
"Extra-pair Male visits"	*The number of times a male from outside of the pair bong was observed "on" the nest.*	Authors' definition
PAIR BEHAVIOUR		
"Antagonistic behaviour"	*Any aggressive behaviour by the social parents directed towards each other.*	Authors' definition
"Duet"	*Male and Female of the pair singing in association with each other, at the same time.*	Slater & Mann, 2004

3.3.2. Statistical Analysis

The effect of brood size (1 – 4 nestlings) on parental investment was tested using ANOVA examining variation in the number of visits per hour, length of time on the nest and length of time between visits. Where there was no effect of brood size on the attendance of adults in categories tested (See Section 3.4.1.1), data from various brood sizes were combined. The effect of brood sex ratio was tested using Spearman's rank correlations between the proportion of males in a nest and feeding rate (visits per hour). Data were tested for normality using Shapiro-Wilks test (SAS Institute, 2003). Non-normal data were transformed using logistic (log+1) transformations as suggested by (Zar, 1974). Where transformations failed to normalise the data or where samples sizes were small (n>5), non-parametric tests were utilised.

Differences in male and female behaviour at various nest stages were tested by two-way ANOVA (SAS Institute, 2003) (including interactions). Behaviours within a nest stage were compared separately for the sexes using student's t-tests or the normal approximation of the Wilcoxon-Mann-Whitney-U test with correction for continuity (SAS Institute, 2003) dependent on normality.

Nesting and territorial behaviours were quantified as a percentage of the total time per 1-hour nest watch (as in Clarke, & Clarke, 1999). Nesting behaviour included time (minutes) on the nest and time (minutes) spent approaching/guarding the nest (within 10m). Territorial behaviour was analysed as all behaviour relating to defence of the territory including singing and chasing other birds. Variation in individuals was compared to the individual with the highest values (i.e.: the 'best' individual = 100%).

Variation from the best individual female and individual male (in percentage of an hours' nest watch allocated to nesting behaviour) was tested for correlations with nest cover using Spearman's Rank Correlations (SAS Institute, 2003). Correlations between behaviours, territory characteristics and intruders were made using Spearman's Rank Correlations (SAS Institute, 2003). Territories were mapped and numbers of adjacent territories were counted as well as the territory distance from a feeder (i.e.: how many territories an individual would have to cross to reach the feeder).

3.4. Results

3.4.1. Parental Care

3.4.1.1. The effect of brood size and sex ratio

There was no significant difference in the frequency (F=2.12, df=2, *P*=0.14), duration (Kruskal Wallis χ^2= 3.6, df =2, *P*=0.308) or interval between visits (Kruskal Wallis χ^2=0.427, df=2, *P*=0.8) across varying clutch sizes. Therefore, parental care (as measured here) did not differ with increasing brood size.

The sex ratio of a brood was also not significantly associated with parental care. There was no significant association between the total frequency of visits (males and females combined) for nests with varying proportions of male nestlings in a brood (r_s=-0.38, n=15, *P*=0.15). The proportion of male nestlings in a brood was not associated with male feeding rate (r_s= -0.12, n=15, *P*=0.66) or female feeding rate (r^2=-0.258, n=15, *P*=0.352).

These results demonstrated that parental care of broods, as defined by frequency, duration and interval off nests, was not influenced by brood need. Therefore, for all subsequent results data from all broods were pooled, regardless of size and sex ratio compositions.

Total parental care (male and female combined) varied over the nest stages. Average nest attendance for males and females combined is presented in Figure 3.1. Frequency of visits differed significantly across nest stages (F_3=3.37, *P*=0.02), due to incubation. Likewise, duration of visit varied significantly among nest stages (F_3=5.50, *P*=0.002) due to the length of incubation bouts.

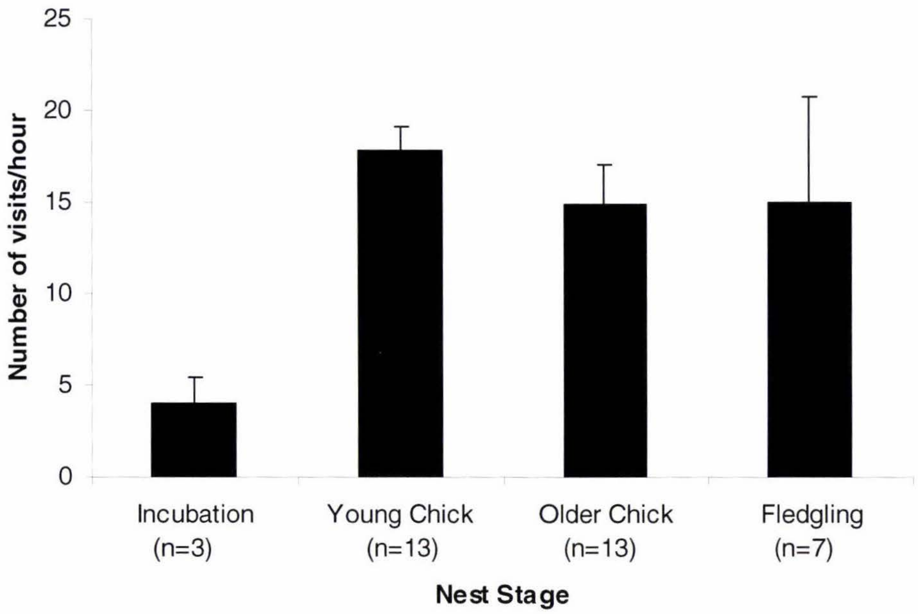


Figure 3.1: Average number of nest visits per hour at each nest stage for males and females combined. Sample sizes are represented under each stage. Bars represent standard error values.

3.4.1.2. Differential investment between parents

Females were solely responsible for nest building and incubating. Incubation bouts ranged from approximately 8min to 45min ($\bar{x}$=22 ± 13min S.E.), while intervals off the nest were approximately 5min (± 0.66 min S.E.). Males made approximately one visit to the nest per hour during incubation ($\bar{x}$=0.833 ± 0.288 S.E., n=3), either to feed the female or call her off the nest.

Similarly, after the eggs hatched, males and females differed in their levels of investment in the nest. The percentage contribution of males ranged from zero to sixty percent, with a mean of 36%. Females contributed significantly more to nest attendance than males (Wilcoxon W=376, Z approximation =-5.72, n=26, P <0.001), averaging 64% of contribution and ranging from 40 to 100% of all contributions to a nest. Females visited the nest more frequently (see Table 3.2 and Figure 3.2) ($t_{2,0.05}$ = 1.66, df = 70, P = <0.001), for longer (see Figure 3.3) ($t_{2,0.05}$ = -2.34, df = 70, P = 0.02) and with less interval between visits (see Figure 3.4) ($t_{2,0.05}$ = 2.16, df = 70, P = <0.001).

Both adults fed the nestlings. Females visited younger chicks more frequently at a rate of 12.76 times (±1.35 S.E.) per hour, compared to males who visited on average 6.8 times (±0.79 S.E.) per hour ($t_{2,0.05}$ = -2.57, df=24, P=0.01; Figure 2). The duration of female visits to younger chicks (1.85 minutes per visit ± 0.31 S.E) exceeded that of males (0.21 minutes per visits ± 0.01 S.E) and was statistically different (W=91, Z=-4.3, P=<0.01) than males (see Figure 2), possibly due to the thermoregulation needs of young chicks and the female brooding role. Older chicks were attended to significantly more frequently by females (Wilcoxon W=132, Z approximation =-2.2, n=13, P =0.02) at a rate of 9.6 visits per hour (±1.31 S.E.) and 5.1 visits per hour (±1.17 S.E.) by males.

Both sexes attended fledglings equally in number of visits ($t_{2,0.05}$ = -0.91, df = 12, *P*=0.379), length of visits ($t_{2,0.05}$ = -0.45, df = 9, *P* = 0.65) and interval between visits ($t_{2,0.05}$= 0.30, df= 12, *P* = 0.76). Males attended fledglings at a rate of 4.86 (±1.59) times per hour, for a mean period of 0.08 (± 0.01 S.E.) minutes per visit with an absence of 7.28 (±0.48 S.E) minutes between visits. Females attended fledglings at a rate of 10 (±5.4) visits per hour, for 0.08 (± 0.03 S.E.) with an average absence of 12.41 (±7.21 S.E.) minutes between visits.

Table 3.2: Number of nest visits per hour at each nest stage showing average ± standard error.

Stage	Nest watch hours	Total Visits/hour	Male visits/hour	Female visits/hour
Incubation (n=3)	**5**	4 ± 1.4	0.83 ± 0.2	3.17 ± 1.24
Chick Young (n=13)	**13**	17.8 ± 1.26	6.85 ± 0.78	12.77 ± 1.35
Chick Older (n=13)	**13**	14.8 ± 2.2	5.1 ± 1.16	9.61 ± 1.32
Fledgling (n=7)	**7**	15 ± 5.7	4.8 ± 1.6	10.14 ± 5.48

There was a significant difference between duration of visits (F_3=5.61, *P*=0.01) and interval between visits (F_3=3.20, *P*=0.02) by each sex over all the nest stages. However, there was no difference between males and females in the frequency of visits across different nest stages (F_3=0.19, *P*=0.90). The number of times a male visited a nest was positively correlated with the number of times a female visited a nest (r_s=0.43, *P*=0.001, n=16, Figure 3.5)

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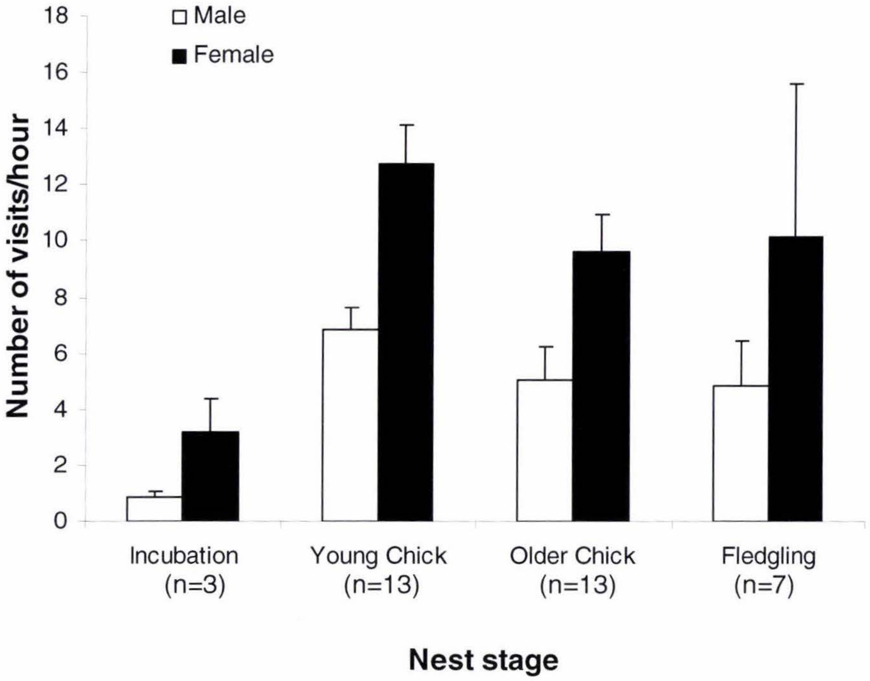


Figure 3.2: Average number of visits by male and female Bellbirds per hour. Sample sizes are represented under each stage. Bars represent standard error values.

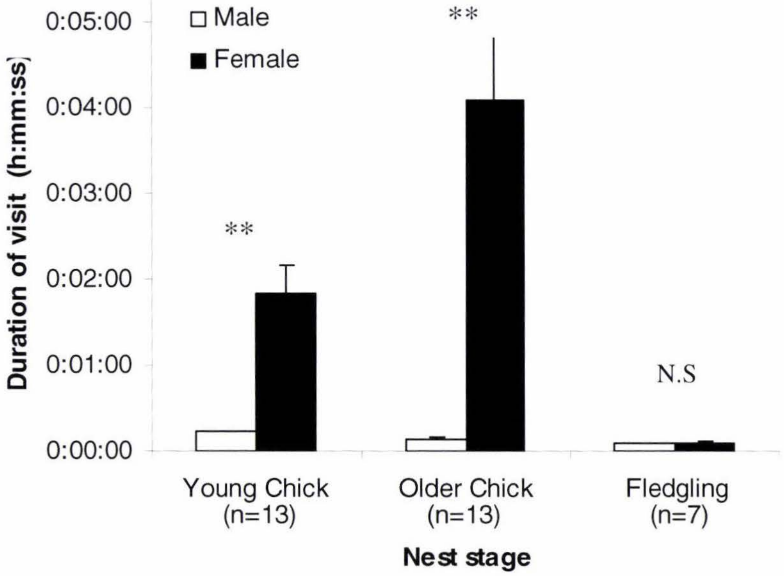


Figure 3.3: Average length of time on nest for each visit per hour for chicks and fledgling stages. Incubation period not shown since scale is much larger and consists of only female data. Sample sizes are represented under each stage. Bars represent standard error values. ** denotes $p<0.01$, N.S= non-significant ($\alpha=0.05$).

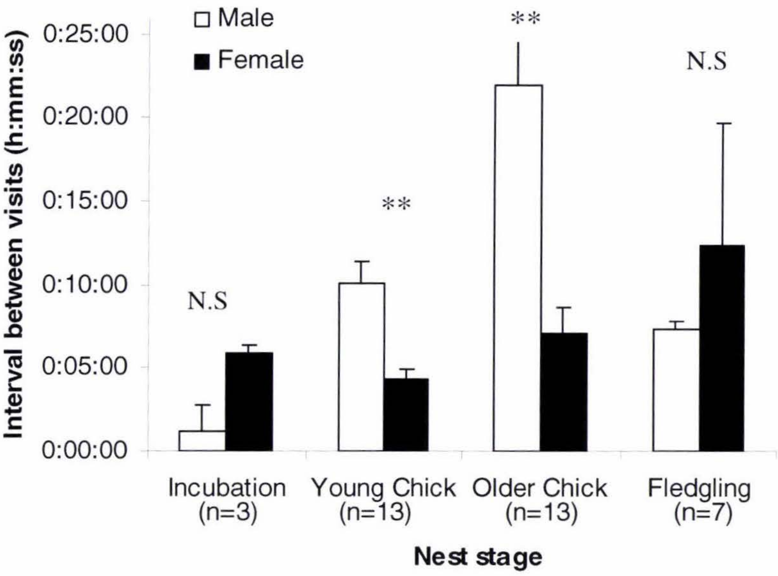


Figure 3.4: Average length between nest visits per hour for male and female Bellbirds at each nest stage. Sample sizes are represented under each stage. Bars represent standard error values. Differences between males and females at each stage were tested using students t-tests, ** denotes p<0.01, N.S= non-significant (α=0.05).

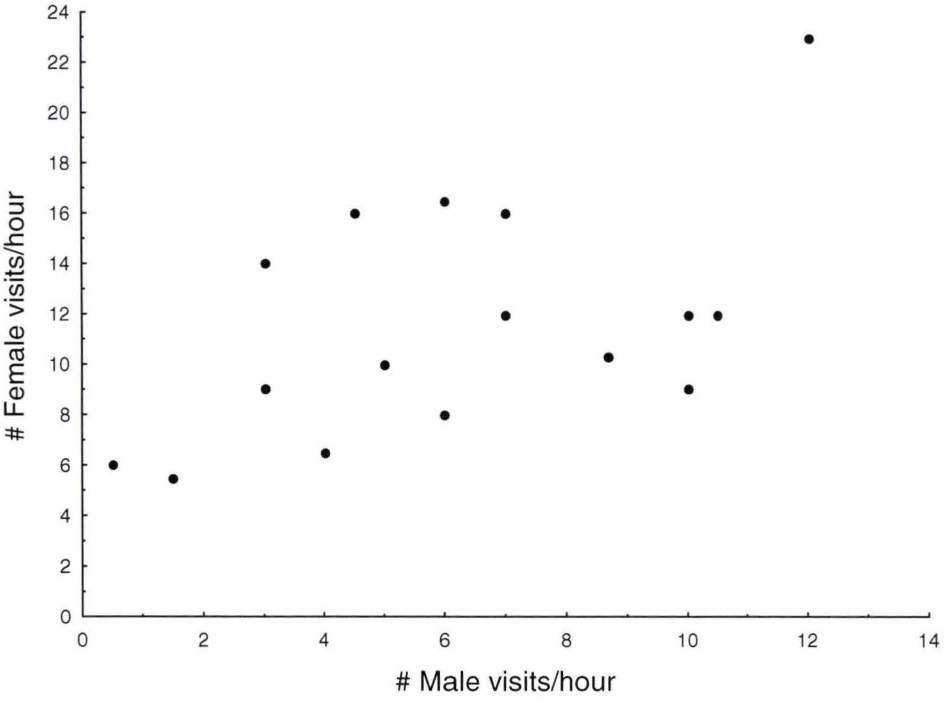


Figure 3.5: Association between frequency of male visits per hour and female visits per hour during the chick stage (n=16).

3.4.1.3. Individual Variation

Individuals varied in the level of investment for each nest. There was up to 36% difference in time allocation to nesting behaviour between individual females and up to 26% in individual males (See Figure 3.6). Variation in male and female time allocated to nesting behaviour was not correlated with condition, measured as adult weight:tarsus ratio (male: r_s=0.07, n=12, *P*=0.82; female: r_s=-0.13, n=12, *P*=0.68), or the percentage cover over a nest (male: r_s=-0.26, n=12, *P*=0.4; female: r_s=-0.30, n=12, *P*=0.34).

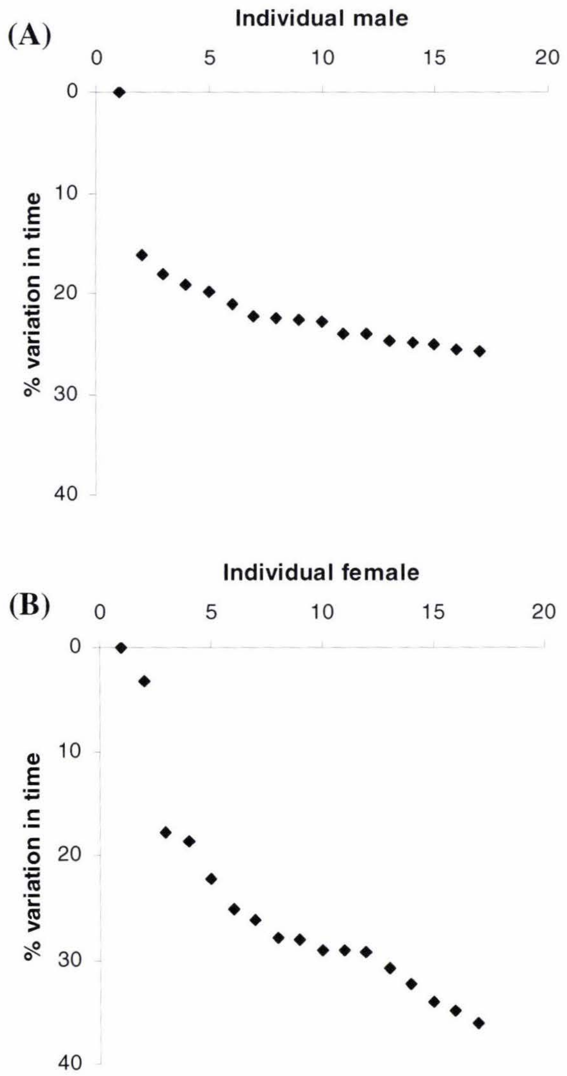


Figure 3.6: Deviation from 'best' female (A) and male (B) in percentage of nest observation time allocated to nesting behaviour.

3.4.2. Social interactions

The majority of pairs (92%) were observed to remain together for the duration of the breeding season and subsequent years. Only two cases of divorce were observed, both of these occurred between breeding seasons rather than within a breeding season. In one instance the resident male (M-YG) was usurped by a 'floater' male (RW-M) from the previous year. In the second instance the resident male (OM-G) was not seen again and was presumed dead. The usurped male (M-YG) from the previous nest replaced this male (OM-G).

Territorial behaviour included defence (song and chases), pair co-ordination (duetting) and territorial encroachments or forays. Males undertook 1.2 (± 0.3 S.E.) chases per hour, while females undertook 0.7 (±0.2 S.E.) chases per hour. There was no significant difference in the number of chases undertaken by male or female Bellbirds ($t_{2,\ 0.05} = 1.27$, DF=34, $P=0.21$). Males sang on average 3.2 (±0.8 S.E.) times per hour while females sang significantly more frequently ($t_{2,\ 0.05} = 3.64$, DF =34, $P=<0.0001$) with an average of 9.6 ± 1.6 (S.E) songs per hour. There was a negative correlation between the number of male songs per hour and the number of male Bellbird encroachments on the territory although this was not statistically significant ($r_s=-0.44$, $P=0.07$, n=18). However, the trend suggests that the more males sang the less territory incursions occurred in his territory. There was a significant positive correlation between number of female songs per hour and the number of female Bellbird encroachments ($r_s=0.55$, $P=0.01$, n=18). The number of female encroachments into a territory was not positively correlated with the number of adjoining territories ($r_s=0.339$, p =0.168, n=18). If detected, territory encroachments resulted in the territory owner chasing; singing and chasing; or physically attacking the intruder; in both sexes. Territory encroachments sometimes included extra-pair males gaining access to the nest (n=8/19).

Extra-pair male visits occurred throughout the nesting cycle. Males adopted a cryptic posture, with slow vigilant movements towards the nest. There was at least one occurrence of a non-territorial male arriving at the nest with invertebrates in its bill, only to be chased by the incubating female. If extra-pair males are attempting extra-pair fertilisations, one would expect there to be a decrease in visits after incubation. However, there was no statistical variation in extra-pair male visits over nest stages (r_s=0.02, *P*=0.9, n=36) despite the observed trend of decreasing visits over time (Figure 3.7). There was one apparent case of polygamy where the social father of "older" stage chicks at a primary nest was defending a female building/laying on the edge of his territory. That same primary nest was frequented by two individually identified males, one of which mate guarded and defended the nest throughout nesting and the other which fed the chicks. This indicates a possible case of polyandry and polygyny at the same nest (confirmed through molecular analysis of paternity, See Chapter four). In one instance, mist netting within a territory of a fertile female resulted in six different males (four of which were identified as adjacent neighbours), and two unidentified females being caught.

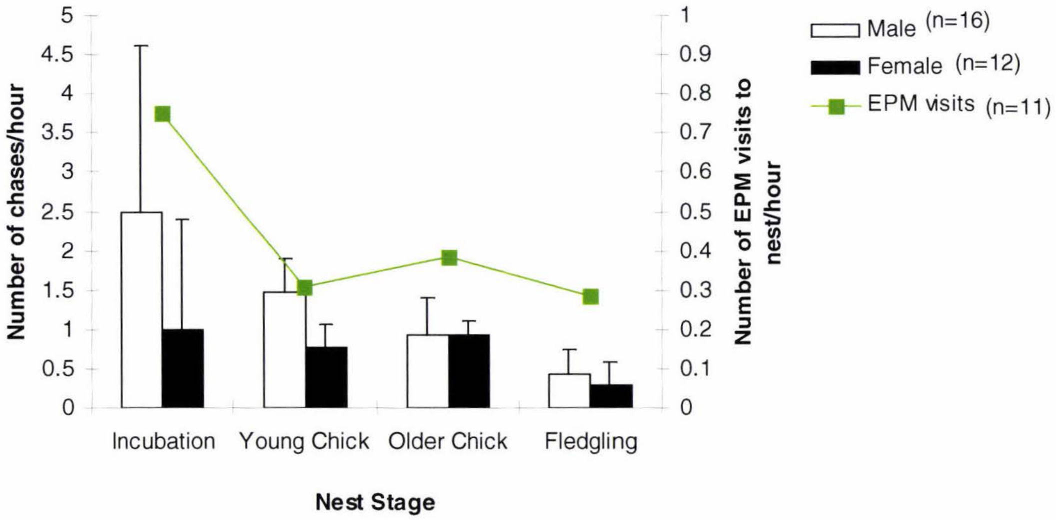


Figure 3. 7 Frequency of male and female chases per hour at each nest stage, showing the corresponding rate of extra-pair male (EPM) visits to nests at those stages.

Territories were approximately 100m^2 during the breeding season and were in close proximity of neighbouring pairs (See Figure 3.8). Bellbirds were seen chasing conspecifics within a radius of 10m from the nest site. Each pair held a distinct area in which other Bellbirds were excluded; these ranged in size, however, remained relatively stable between years. Pairs had high site fidelity with 78% returning to the same area each year and included five cases of the same tree being used as a nest site in subsequent years.

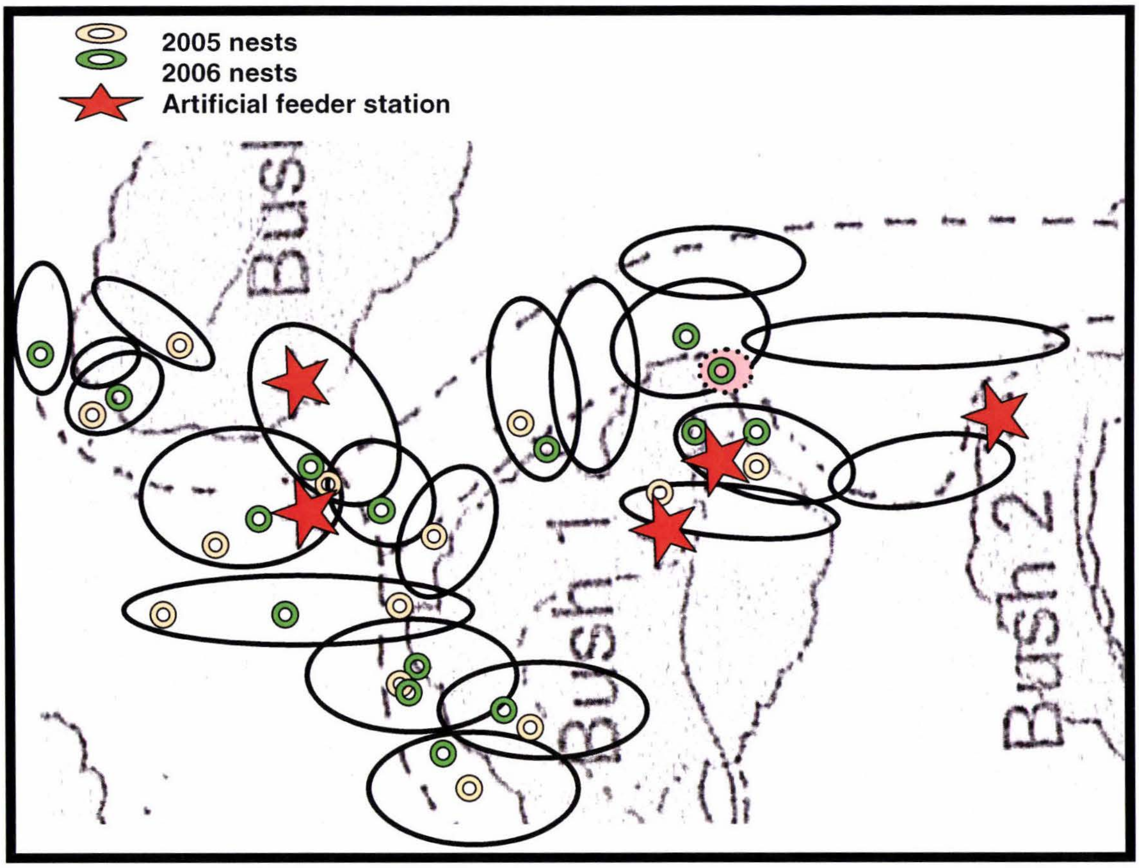


Figure 3. 8: Territories of Bellbirds within the study site. Each individual circle represents a territory held by a Bellbird pair throughout the breeding season. The polygynous nest and territory is indicated with dashed lines and highlighted pink. Location of nests within each territory for 2005 and 2006 and distance of territory from feeder are shown. Territory size is relative according to observations and is not a direct measure.

Territory proximity to a feeder did not appear to correlate with variables influential on reproductive success (such as intruders) or nest success. Number of encroachments was not related to territory distance from feeder (female encroachments: r_s=0.24, *P*=0.322, n=36; male encroachments: r_s= 0.220, *P*=0.41, n=36). Territory density appeared to increase away from feeders as there was a positive correlation between number of overlapping territories and increasing distance from a feeder (r_s=0.45, *P*=0.07, n=18). There was no correlation between distance from a feeder and nest success (defined as the probability of fledging at least one young) (r_s=-0.15, *P*=0.37, n=18). Three copulations were observed during this study. Two of these were extra-pair copulations (female copulated with a male that was not her social partner) and occurred in territories containing a feeder.

3.5. Discussion

3.5.1. Parental Care

Parental care in Bellbirds involved nest building, incubation, nestling provisioning, fledgling provisioning and nest guarding. Parental care was biparental but female biased. As such, these findings support those of Anderson & Craig (2003) for Bellbirds nesting on Tiritiri. However, mate fidelity between years was higher in this study (92%) than that of Anderson & Craig (2003) (38%). This may have been due to the presence of predators (Kiore) on the island during their study or could be due to the differences in sample sizes between the two studies (this study: n=18 pairs, Anderson & Craig (2003): n=8 pairs). In addition, male 'strangers' were regularly observed visiting nests of Bellbird pairs in this study, where this behaviour was not reported by Anderson & Craig (2003).

Male parental care has been shown to be beneficial in producing successful offspring. Removal of Dark-eyed Junco (*Junco hyemalis*) males during the chicks stage resulted in no effect on the nestlings, but a significant effect on the survival of fledglings (Wolf, Ketterson & Nolan, 1988). The role of male parents varies widely across bird species but is usually less than that of the female (Trivers, 1972). Although this pattern was evident in this Bellbird population, it is interesting to note that female Bellbirds showed more variation in the time allocated to nest attendance than did males. A similar trend of lower variability in male investment has been seen in the House Sparrow (*Passer domesticus*) where males were found to be less variable in their feeding rate than females both within and between years (Nakagawa, Gillespie, Hatchwell & Burke, 2007). Suggestions for this pattern include male advertisement where male parental care may itself be advertisement for future parental care and therefore may influence future mating success (Nakagawa *et al.*, 2007). The high level of mate fidelity in this population may be interpreted as support for this suggestion, as mates tended to stay together between years. However, the two cases of divorce that were observed in this study did not include the 'worst' males (those that indicated the least amount of attendance effort).

Investment in young is costly to an individual (Williams, 1966). This has been shown in many species resulting reduced survival or future reproductive success (e.g.: Bewicks Swans, *Cygnus columbianus bewickii*, Scott, 1980). A trade-off therefore exists as each parent is selected to maximise its remaining or future reproductive success while simultaneously attempting to maximise current reproductive success (Williams, 1966). Parental care is therefore expected to vary according to environmental conditions (Carlisle, 1982) as well as the age, condition and life history of an individual (Kempenaers & Sheldon, 1997). Variation in individual condition has been shown to affect breeding parameters (e.g.: sex ratio allocation; Whittingham & Dunn, 2000). However, in this study there was no association between individual Bellbird condition and percentage time allocated to nesting behaviour. The age of individuals was unavailable for this study however future research should involve monitoring of this population over an extensive period to determine any differences between old and young birds.

Predation is the greatest factor influencing nest failure in most birds (Ricklefs, 1969). Nest attendance has shown to be related to the apparent predation risk in North American passerines (Conway & Martin, 2000). Nest cover is therefore expected to represent the relative risk of predation in Bellbirds. However, there was no association between level of cover over a nest and adult attentiveness.

Research has shown that the amount of energy invested in young is expected to decline with declining resources (Clutton-Brock, 1991). The timing of breeding may have influenced the amount of parental investment through the quality of food provided to chicks or through fewer visits. Due to limited sample sizes all nest watches were pooled. Therefore, it was not possible to detect any such impact of changing seasonal resources on parental investment within the scope of this study. It is thus recommended that future research should incorporate investigating resource levels within territories to determine the effect on parental investment.

The sex ratio of a brood can indicate the need of that brood through unequal growth in dimorphic species resulting in a larger amount of food being required to support the larger sex (Stamps, 1990). Males are usually more costly to raise (e.g.: Great Tailed Grackle, *Quiscalus mexicanus,* Teather & Weatherhead, 1988; and Red-winged Blackbird, Fiala, 1981). Bellbirds are dimorphic in size and nestlings show differential growth rate between the sexes (see Chapter two). Therefore, higher provisioning of the faster growing and larger sex could be expected (Stamps, 1990). However, there was no association between the proportion of males in a nest and parental feeding rate.

Brood size is also predicted to reflect brood need. For instance studies of male Field Sparrows (*Spizella pusilla*) showed that males adjusted parental care based on brood size in terms of numbers of visits as well as prey size (Carey, 1990). In this study adult Bellbird parental care, as measured by frequency of visits, did not vary with brood need. However, it must be acknowledged that the size of prey items brought to nestlings was not measured in this study. There is therefore the possibility that adults increased parental care through the provisioning of larger prey items or higher quantities of food.

Negotiation over offspring care is predicted to show a positive correlation between male and female provisioning rate if the differences in provisioning rate change with brood need (Johnstone & Hinde, 2006). Although adults did not differentially provision nestlings based on brood need, there was evidence for negotiation over offspring care. This is because male and female visits to the nest were positively correlated which supports the negotiation hypothesis in that efforts of one sex are matched by that of the other (Johnstone & Hinde, 2006). This study assumed that all chicks were fed equally and parents were similar in the quantity of food delivered to nestlings. However, parents may be able to reduce the rate of nest attendance by increasing the size or quantity of prey items (as suggested for tropical species; Slater & Mann, 2004).

Parental care is expected to vary greatly according to paternity of the brood (Moller & Birkhead, 1993). Evidence supporting this theory has been demonstrated by experimentally manipulating certainty of paternity in groups of Starlings, where males that had less certainty of paternity showed significantly lower levels of paternal investment in raising the brood (Wright & Cotton, 1994). However, many other studies have shown conflicting results. Male Western Bluebirds did not reduce their level of nestling provisioning when they were detained despite witnessing their female engaging in extra-pair copulations (Dickinson, 2003). Observational studies of Razorbills (*Alca torda*) have also supported the finding that female extra pair copulatory behaviour does not relate to levels of male parental care. In contrast, Westneat & Sargent (1996) theorise that if parents are able to determine paternity of young within a brood then parental behaviour would be directed preferentially within a brood, although no evidence for this behaviour has yet been found. Furthermore, parental care performance in males may not even be related to current parentage within broods, but may alternatively be a product of male advertising for future reproductive success. For example, performance of a male in parental care duties for first clutches of American Robins (*Turdus migratorius*) was related to the proportion he fathered in the second clutch (Rowe & Weatherhead, 2007). The relatively high level of male parental investment by Bellbirds suggests that either there is a high confidence of paternity or males are using parental care as an advertisement for future matings.

If mortality is biased to a particular sex, the evolutionary stable strategy in terms of allocating resources to reproductive success may differ from the strategy employed by the other sex (Trivers, 1972). There is some evidence that survival is lower for female Bellbirds than male Bellbirds on the Poor Knights Islands (Sagar & Scofield, 2006). However, data on survival and longevity of Bellbirds on Tiritiri were not available for this study. Therefore, it is recommended that future studies utilise this large banded population to determine what effect survivorship, adult sex ratios and longevity of this species may have on its breeding system.

3.5.2. Social interactions

Bellbirds generally exhibited a socially monogamous mating system as shown in Anderson & Craig (2003). Mate fidelity was high within and between seasons and one case of divorce occurred as a result of usurpation by another male. However, there was one documented case of polygyny and polyandry occurring in the same territory. Additionally, extra-pair copulations were observed in this population and occurred when the female solicited from an extra-pair male on her territory.

Both male and female Bellbirds were observed defending the nest area against conspecifics. These territory defence behaviours involved chases and singing. Song has been documented as playing a major role in territory defence in many species. Including Nightingales, *Luscinia megarhyncos*, (Naguib, Amrhein & Kunc, 2004); Superb Fairy-Wren, *Malurus cyaneus*, (Cooney & Cockburn, 1995); Alpine Accentors, *Prunells collaris*; and Dunnocks, *Prunella modularis*, (Langmore, 1996). Such defence of breeding territories has been proposed as a tactic for the prevention of cuckoldry (Moller, 1987). The singing rate of male Bellbirds was negatively correlated with number of male encroachments, suggesting that it possibly plays a role in prevention of cuckoldry.

Bellbirds are also unusual in that females not only sing, but they have a complex repertoire comparable to that of males of other passerine species (Brunton & Li, 2006). Recent studies on female Bellbird territoriality and the role of song have indicated that females respond significantly more to playbacks of neighbouring females than those of female strangers (Brunton *et al.*, *in review*). In this study current female singing rate was positively associated with the number of female encroachments onto the territory. This could be considered a threat response, as shown by Brunton *et al.*, (*in review*), where Bellbird females are responding to the number of neighbouring encroachments. However, experimental evidence is needed to determine the cause and effect of increased female singing.

The role of song in female birds is hypothesised as a prevention of polygyny; protection of maternal investment; mate attraction; and for mate stimulation. It has been demonstrated as preventing polygyny and ensuring male parental care in Starlings (Sandell & Smith, 1997) and Red-winged Blackbirds (Yasukawa & Searcy, 1982). It has also been suggested to play a role in resource defence (Berglund, Magnhagen, Bisazza, König & Huntingford, 1993), however this would result in aggressive encounters between females and all conspecifics (not specifically neighbouring females). The protection of maternal investment can also involve protecting the nest from being destroyed by conspecifics and protection from intra-specific brood parasitism. For instance, Starling females have been shown to exhibit nest defence against other female starlings as this species has been shown to engage in intraspecific brood parasitism (Evans, 1983). Langmore *et al.,* (1996) showed that intrasexual female competition in the Alpine Accentor occurred as a result of competition for access to males (mate attraction). The hypothesis of mate stimulation was suggested by Dilger (1953) after observing courtship and duetting in the Crimson Barbet (*Magalaema haemacephala*).

Female Bellbirds sing year round (Brunton & Li, 2006) including the period of winter flocking with other females and juveniles. However, females sing more frequently in the breeding season than males (Brunton & Li, 2006) and direct their territorial aggression toward neighbouring females (Brunton *et al.*, *in review*). The current evidence available for the role of Bellbird female song does not fit into the current theories for the role of song in female birds (presented in Table 3.3). It is unlikely to be as a result of purely resource protection, as this would result in aggressive encounters between females and all conspecifics (not specifically neighbouring females). Further experimental evidence of the behaviour of females to male song playbacks within her territory may clarify whether the function is for resource defence. However, it may be possible that a combination of the hypotheses will be needed to explain song function in female Bellbirds. The possibility of intraspecific brood parasitism (IBP) in this population of Bellbirds is discussed in Chapter 4. Future research including mate removals, positioning of mounted female specimens near the nest and artificial brood parasitism (by placing an extra egg into the nest) would increase the current understanding of Bellbird social dynamics.

Table 3.3: The hypotheses formulated for the role of female song and their expected behaviours with the current evidence for the role of female song. 1 (Yasukawa & Searcy, 1982) 2 (Berglund *et al.*, 1993) 3 (Liker & Székely, 1997) 4 (Davies *et al.*, 1992) 5 (Langmore, 1996) 6 (Dilger, 1953).

EXPECTED BEHAVIOURS	**Aggression aimed at:**			**Timing of aggression :**			
	Males	**Females**		**Breeding Cycle**			**Throughout Year**
HYPOTHESES		Neighbours	Strangers	Peaks Early	Increases	Constant	
Competition for Male Parental Care[1]		✓		✓			
Resource Defence[2]	✓	✓	✓			✓	✓
Nest Protection[3]	✓	✓			✓		
Preventing IBP[4]		✓		✓			
Mate Attraction[5]	✓					✓	✓
Mate Stimulation[6]	✓			✓			
Bellbird Evidence		✓				✓	✓

Male song is involved in male-male competition (Ligon, 1999) and can be used in a sexual context, playing a role in female choice (Mountjoy & Lemon, 1996) and also attraction (Eriksson & Wallin, 1986). Male Bellbirds regularly sing and display in groups (Brunton & Li, 2006). These display groups are termed 'corroborees' in many of the Australian honeyeater species (Pyke & O'Connor, 1989). These displays are initiated by individual male Bellbirds and result in the attraction of females to the area (pers.obs). The role of these aggregations is uncertain and future research needs to determine the context, resource availability and outcome of these clusters to determine the possibility of hidden leks (Almeida & Macedo, 2001) in this species.

Extra territorial forays are common in some bird species (e.g.: Yellow-breasted Chat, *Icteria virens*, Mays & Ritchison, 2004) and territory intrusions were relatively common in this Bellbird population. Males were more conspicuous than females in extra-territorial behaviour and hence were detected more frequently. However, only males were observed approaching or entering foreign nests. In other honeyeater species (e.g.: Brown-backed Honeyeater (*Ramsayornis modestus*) and Crescent Honeyeater (*Phylidonyris pyrrhoptera*) extra-pair males have been known to frequently visit nests (Maher, 1988; Clarke & Clarke, 1999). The result of these visits has generally involved the visiting adult pecking the nestlings to death and has been suggested as infanticide (Clarke & Clarke, 1999). No cases of infanticide were observed in any of the eight extra-pair male visits to the nest. The majority of males seemed to be assessing the contents of the nest, unprovoked or after a disturbance to the nest. There was one documented case of infanticide by another honeyeater species, a Tui, which was nesting in the vicinity.

The availability of a constant nectar source for a territorial, nectivorous species is likely to have consequences on the social dynamics of the birds in the vicinity. Dominant male Bellbirds have been documented as holding exclusive feeding territories (Craig, 1985). However, demand for resources during the energetically costly nesting season (Williams, 1966) may influence the patterns of territory encroachments in those containing a feeder.

The occurrence of out of season copulations (termed ‘prostitution’ by Wolf, 1975) for access to resources has been documented in female Bellbirds (Craig, 1985). Indeed one of the extra-pair copulations observed in this study occurred at a feeder. The frequency of this behaviour is unknown. However there is the potential for individual dominant males to have an increased frequency of interaction with females and thereby potentially influence paternity if these interactions occur during female fertile periods. In this study, territory encroachments did not differ with proximity to feeders. Importantly, Bellbirds did not regularly use feeders during the breeding season when other sources of nectar are available (pers.obs). However, distance to the nearest natural nectar source may be a more reliable indication of territory quality during the breeding season and the effects should be investigated further.

3.6. Conclusion

This study reports the first documented case of extra-pair copulations and extra-pair territorial encroachments/forays in Bellbirds. Although parental care was biparental, males invested far less in attending the nest than did females. However, variation in time allocated was not related to brood need, nest exposure or adult condition. Since male parental care is predicted to vary with certainty of paternity, the level of extra-pair paternity in each nest needs to be investigated to determine the cause of the large intersexual variance in nesting behaviour. The role of song in female Bellbirds is likely to involve several aspects of defence; however due to the large variation in nest attendance in females, maternity of clutches should also be investigated.

CHAPTER FOUR: Genetic Investigation of Paternity.

Bellbird nestlings.
Photo: Taneal Cope, 2006

4.1. Abstract

Minisatellite DNA fingerprinting was used to assign parentage in 24 nestlings from 14 Bellbird nests on Tiritiri. Analysis revealed that extra pair young were present in 92% of all broods. A total of 81% of offspring were the result of extra pair paternity (EPP). The frequency of EPP detected in this study suggests that Bellbirds have a highly promiscuous mating system that is not reflected in their social bonds. Three potential cases of intraspecific brood parasitism were detected and are discussed in this Chapter. Evidence from this research suggests that both male and female Bellbird engage in mixed reproductive strategies. Therefore, their reproductive success differs from that predicted by the socially monogamous pair bonds. Results also indicated high levels of background bandsharing in this population that were comparable to that of Hihi on Tiritiri, a species that has undergone a significant population bottleneck.

4.2. Introduction

In sexually reproducing species, females produce larger gametes than males (Krebs & Davies, 1991). This unequal division of investment by the sexes means that females usually become a limiting resource for males (Krebs & Davies, 1991). Trivers (1972) argued that this anisogamy leads to a) males behaving in ways so as to increase the number of matings with females and b) females choosing specific males based on the resulting direct and/or indirect benefits to herself or her offspring. This principle was the basis of Charles Darwin's (1871) theory of sexual selection where males compete with one another for access to females and females choose particular males as mates. Despite the realisation of male-male competition and female mate choice, most early observational studies concluded that bird species were largely monogamous (~90%) (Lack, 1968). However, the development of genetic techniques to assign parentage revolutionised our view of avian mating systems and has clearly shown we cannot determine mating system patterns based on observation. It is now commonplace to discover polygamous mating behaviours in species that are considered socially monogamous (Birkhead & Moller, 1996).

Variations from social monogamy are measured via analysis of the genetic mating system that is typically quantified as the frequency of extra pair young (EPY) (Bennett & Owens, 2002).

The discovery of hypervariable regions of 'minisatellite' DNA by Jeffreys, Wilson & Thein (1985a) in humans meant that individuals could be identified based on the genetic pattern of this region. Minisatellite regions consist of neutral DNA, made up of repeated units each of 15 – 60 base pairs (Jeffreys, Wilson & Thein, 1985b). These regions show great variation in the number of repeated units and occur throughout an individuals' genome (Jeffreys *et al.*, 1985a). However, there tends to be a shared 'core sequence' in minisatellites, which means that many different sites of hypervariable regions can be detected by using this 'core sequence' as a probe (Jeffreys *et al.*, 1985a). The hybridisation of these probes to an individuals' DNA can result in a unique pattern of bands evident on a radiograph, known as a 'fingerprint' (Jeffreys *et al.*, 1985b). Each minisatellite banding pattern is unique to an individual or identical twins and is inherited in a mendelian fashion. Mendelian inheritance of genes specifies that each individual inherits half its genome from each parent, therefore the combination of each parents' 'fingerprint' should be able to explain all bands present in an offspring.

In addition to their ability to provide individual specific genotypes of people, the same DNA fingerprinting probes (in particular Jeffrey's 33.15 probe) have proven extremely useful in their ability to detect high variation in other organisms (Lambert & Millar, 1995) including birds (Burke & Bruford, 1987). This method of DNA fingerprinting has been widely used in studies of biological relationships in populations (e.g.: Burke & Bruford, 1987; Westneat, 1990; Wetton, Carter, Parkin & Walters, 1987; Birkhead, Burke, Zann, Hunter & Krupa, 1990; Miller, Lambert, Millar, Robertson & Minot, 2003). As such, DNA fingerprinting techniques have provided proof that extra-pair parentage occurs regularly in populations, either through extra-pair paternity (e.g.: House Sparrow: Wetton *et al.*, 1987; Hihi: Ewen *et al.*, 1999) or intraspecific brood parasitism (e.g.: Zebra finches, *Taeniopygia guttata*: Birkhead *et al.*, 1990).

Trivers (1972) suggested that males could increase their reproductive output by engaging in a mixed reproductive strategy of caring for the offspring of his monogamous mate while not passing up the opportunity to fertilise other females, thereby taking advantage of another individuals' parental care. Comparatively little attention has been given to female reproductive strategies and the choices that females make throughout breeding. The choice of where to nest does not necessarily reflect the mating strategy (whom to mate with) in female birds (e.g.: Red-winged Blackbirds: Gibbs, Weatherhead, Boag, White, Tabak & Hoysak, 1990). In socially monogamous species the random variation in individual quality of mates means that not all females are mated to a preferred male (Moller, 1999). Females may have to accept social bonds with lower quality males in order to secure a breeding site and some level of paternal care but seek extra pair copulations to receive the genetic benefits of superior males within a population.

Most species of birds that were once considered monogamous have now been shown to engage in extra-pair copulations (EPCs), the rate of which varies among species (see Birkhead and Moller, 1992, for an extensive summary). Many species exhibit 'cryptic polygamy' where socially monogamous bonds show a continuum of extra pair fertilisation (EPF) rates (Johnson & Burley, 1997) known as mixed reproductive strategies (Trivers, 1972) that can enhance both a males' and females' reproductive success (Ewen *et al.*, 1999); Gibbs *et al.*, 1990).

Females may engage in EPCs to gain direct and/ or indirect benefits to herself or her offspring (Jennions & Petrie, 2000). Direct benefits include access to resources, nest and territory defence and increased predator vigilance (Birkhead & Moller, 1992). In addition, paternal care can be an important direct benefit as it may result in higher quality offspring with increased survival after fledgling (Wolf *et al.*, 1988). Indirect benefits of female choice are usually manifested through the genetic contribution of extra-pair males. For example, females may choose genetically similar males due to beneficial interactions between compatible genes or genetically dissimilar males to increase the level of heterozygosity in her offspring as a measure of inbreeding avoidance (Foerster, Delhey, Johnsen, Lifjeld & Kempenaers, 2003). The genetic diversity hypothesis suggests that in environmentally unstable conditions, variation in offspring phenotypes can increase the chance of successful reproduction (Williams, 1975). In addition, females may choose males based on their phenotypic quality (Weatherhead & Robertson, 1979) in order to increase her future contributions to the gene pool by producing high quality offspring. Male Bellbirds differ in dominance and therefore access to resources (Craig & Douglas, 1986) as well as differing in size (See

Chapter two), which may have potential implications for female mate choice in this system.

A review by (Moller & Birkhead, 1993) found that variation in paternal care is related to certainty of paternity. In addition, species where the male plays a relatively insignificant role in rearing the offspring are often characterised by high levels of EPP (Moller & Cuervo, 2000). It is interesting to note that male and female Bellbirds differ significantly in the extent of parental care with males investing far less in brood rearing compared to the female (See Chapter three). Furthermore, species that show a large degree of sexual dimorphism generally show a high level of promiscuity in their genetic mating system (Owens & Hartley, 1997). For example, the Splendid Fairy Wren *Malurus splendens* (Webster, Tarvin, Tuttle & Pruett-Jones, 2007), Hihi (Ewen *et al.*, 1999), Blue-black Grassquit *Volatinia jacarina* (Carvalho, Macedo & Graves, 2006) and Mallard ducks *Anas platyrhyncos* (Evarts & Williams, 1987), all exhibit high levels of sexual dimorphism and high levels of EPP in broods. Bellbirds also exhibit substantial sexual dimorphism in size and plumage; therefore the genetic mating system could be predicted to show high levels of polygamy through extra-pair fertilisations. Additionally, the observations of social interactions including extra-pair copulations near food sources (See Chapter three) suggests that paternity of clutches, especially adjacent to artificial feeders, will not reflect the socially monogamous pair bonds present.

Bellbirds present an interesting case for the investigation of genetic relationships. They exhibit a large level of sexual dimorphism (See Chapter two), unequal parental care (discussed in Chapter three), dominance hierarchies in access to resources (Craig &

Douglas, 1986) and females focus their territorial aggression toward other females (Brunton *et al.*, *in review*). The investigation of genetic paternity in Bellbird broods may serve to further understand the unique behaviours of this species.

4.2.1. Objectives

The primary aim of this chapter is to document paternity in broods of Bellbird chicks sampled in 2005 and 2006 to determine the presence of extra-pair paternity.

Specifically, I am to:

1. Document the presence of extra-pair parentage in this population of Bellbirds. This will be assessed using minisatellite DNA fingerprinting thereby:
    - Determining the occurrence of extra-pair paternity.
    - Determining the presence or absence of intraspecific brood parasitism
2. Document the proportion of young sired by extra-pair males.
3. Determine the association of extra-pair paternity with individual variation in paternal care.
4. Determine the association between the percentage of extra-pair young and variation in male behaviour including territorial defence and individual condition.
5. Determine the association between territory intrusions and territory distance from feeder on the rate of EPP in broods.

4.3. Methodology

4.3.1. DNA preparation

Blood samples were taken from all chicks, embryos (unhatched eggs), parents of each nest as well as neighbouring individuals and any identifiable intruders (See Chapter two methods). DNA was extracted using phenol: chloroform, as in Millar *et al.*, (1992) (See APPENDIX II for details). High molecular weight DNA was suspended in 20-40 μL milli-Q water and stored at 4 C. Twenty μL of DNA (approximately 500 ng) was digested with the restriction enzyme *Hae*III in a solution of 4 μl 10xBuffer (100mM Tris-HCl (pH 7.9), 500 mM NaCl, 100 mM $MgCl_2$ and 10 mM dTT), 2 μl BSA (2 mg/ μL), 1 μl Spermidine (4 mM), 2 μl *Hae*III (0.5 U/ μL) and 11 μl milli-Q water. Incubation of this solution was carried out overnight at 37 C. A further 1 μl of *Hae*III was added in the morning and the solution incubated at 37 C for another hour. Digested samples were labelled and stored at -20 C.

4.3.2. Quantification of digested DNA

The concentration of digested DNA was measured using a Hoefer DyNA Quant 200 fluorometer. One hundred ng/ μl calf thymus DNA was used as a standard to calibrate the fluorometer before testing samples and after every 10 samples.

The quality of the digested DNA samples were checked by running 1 μl of sample on a 1% agarose gel at 100 V for 30 minutes. Each sample was checked for uniformity of smear and intensity. Samples that failed to digest completely appeared as either a single band of high molecular weight or had an uneven streak. These samples were discarded and a new sample of the genomic DNA was digested.

4.3.3. DNA analysis by Southern blotting

Seven µL of digested DNA [0.5 ng/ µL] were loaded in each lane on a 0.8% agarose gel (19x27 cm) in 1xTBE running buffer at 43 V for 48 hours. Samples were loaded in a predetermined order. Bellbird families were grouped together with the putative parents either side of the chicks, within 5 wells of each other. Usually two families were loaded on each gel. Any potential extra-pair males (i.e.: territory intruders and neighbours) were inserted in the middle of the gel so as to compare to both families (See Figure 4.1). The DNA was denatured by washing each gel for 15 minutes in 500 mL of depurination wash (0.25 M HCl) then for 45 minutes in a denaturation wash (0.5 M NaOH, 1.5 M NaCl) followed by 15 minutes in a neutralization wash (1.5 M NaCl, 0.5 M TrisHCl pH 7.2, 1 mM EDTA). Southern blotting (Southern, 1975) was carried out to transfer DNA from the agarose gels to nylon membranes through capillary action in 6xSSC. Nylon membranes were then air dried to remove moisture and baked in tinfoil for 2 hours at 80 C. Baked membranes were soaked in a prehybridisation solution (75 mL 0.5 M Sodium hydrogen phosphate, 75 mL autoclaved distilled water, 300 µl 0.5 M EDTA (pH 8.0) and 10.5 g SDS) for two hours at 65 C.

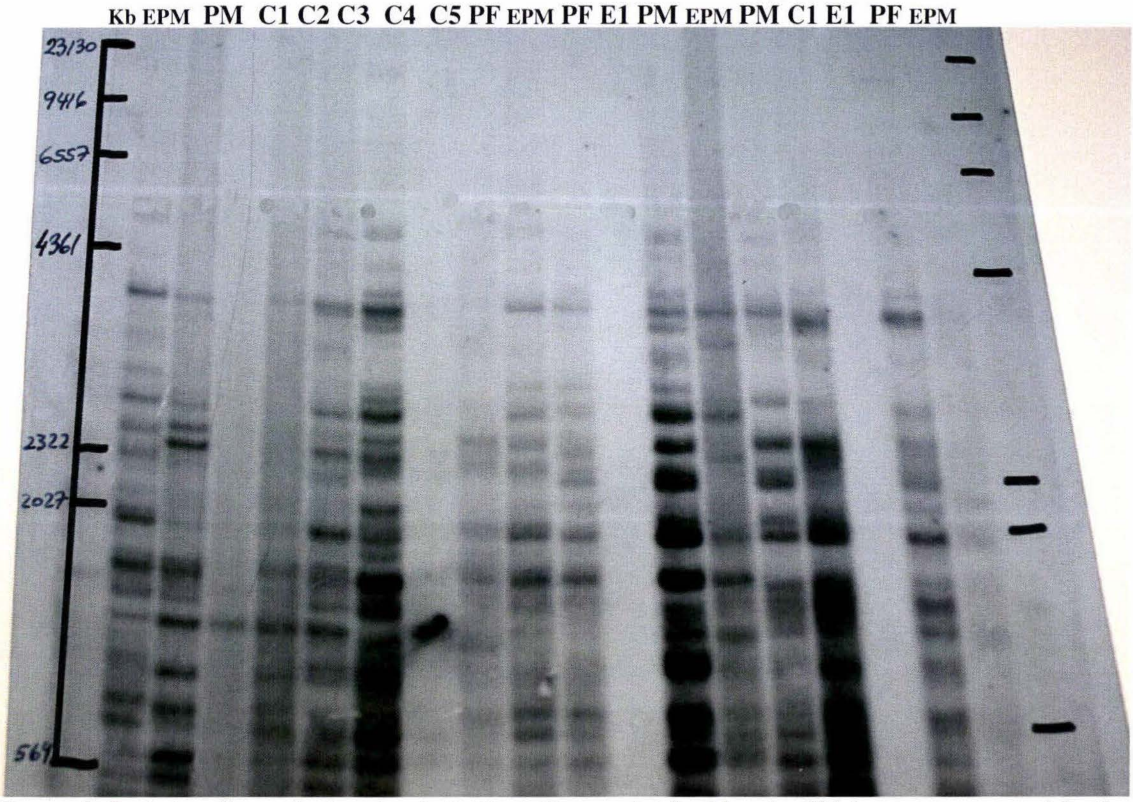


Figure 4. 1: Autoradiograph showing individual ‘fingerprints’ of bands. Chicks are represented in the middle (C1,C2,C3 etc). Putative mothers are represented by PM, putative fathers are represented by PF and extra-pair males are represented by EPM.

Jeffreys 33.15 probe (Jeffreys *et al.*, 1985a) was labelled with α-^{32}P by random priming with Amersham® Random Priming Kit. Any radioactive label that was not incorporated was removed using G-50 Sephadex columns. The probe 33.15 was hybridised to each membrane by incubating at 65 C overnight. Membranes were washed with 5xSSC, 0.1% SDS for 40 minutes at 65 C then exposed on x-ray film at -80 C for seven days. After exposure, membranes were stripped by rocking in 200 mL 0.4M NaOH solution for 40 minutes at 45 C then with 200 mL of neutralising wash (0.1%SSC, 0.1% SDS, 0.2 M TrisHCl) repeated at 45 C for 15 minutes. Each membrane was re-probed with CA probe. CA probe was labelled with α-^{32}P following the methods above. Hybridisation of membrane and the probe CA was at 55 C overnight. Membranes were washed with 3xSSC, 0.1%SDS at 55 C and exposed as with Jeffreys 33.15 probe.

4.3.4. Data Analysis

Each gel was run with molecular markers (λ*Hind*III) in each outermost lane. The markers allowed confirmation of an even run gel and identified band sizes. Bands were recorded on an overlaid acetate sheet (Galbraith, Boag, Gibbs & White, 1991) for each probe separately and for a superimposed combination of each probe (to correct for overlap in probe results). Bands were scored between 5kb – 23kb for both 33.15 and CA probes and were considered identical if there was no more than two fold difference in intensity deciphered by eye and each band occurred with 0.5 mm of each other (Birkhead *et al.*, 1990).

Paternity was assigned based on the presence of novel bands (Westneat, 1990). The presence of novel fragments in offspring has been shown to identify extra pair fertilisations or intra-specific brood parasitism (Westneat, 1990). Considering mendelian inheritance, offspring should inherit half their genome from each parent. Therefore, the combined parental fingerprints should be able to explain all bands present in the offspring (Jeffreys *et al.*, 1985b). This means that bandsharing should be above 50% for first order relatives (Jeffreys *et al.*, 1985b)

Bandsharing was calculated using the formula from (Wetton *et al.*, 1987):

$$D=2N_{AB} / (N_A + N_B)$$

where N_A is the total number of scorable bands in individual A, N_B is the total number scorable in individual B and N_{AB} is the number of bands shared by individual A and individual B (Wetton *et al.*, 1987). Two way comparisons were calculated for each chick and their putative parents (as in Ewen *et al.*, 1999).Total background bandsharing coefficients were calculated as means of all two way comparisons of adults in the population. Chicks were excluded from background bandsharing due to the presence of extra pair sires whose relationship with the chicks would overestimate the levels of bandsharing. Comparisons of related (chicks and genetic parents) versus unrelated (chicks and other adults) individuals were carried out to determine the degree of overlap in bandsharing between first order relatives and other Bellbirds in this population. Tiritiri is a closed island population which could affect background bandsharing. Despite this, adult neighbours were selected as unrelated; assuming a) no natal philopatry and b) the population size is large with the potential of Bellbird immigration from nearby islands.

The probability (p) of false inclusion of a male as the father for each chick was calculated using the following formula:

$$p = \prod_{i=1}^{n} \frac{n_i}{m}$$

Where n_i is the number of males that share the i^{th} paternal band and m is the total number of males included in the paternity analysis (Sarre, Aitken, Clout, Ji, Robins & Lambert, 2000).

Correlations with paternity were assessed using Spearman's Rank Correlations. Only nests that consisted of genetic samples from the complete clutch were analysed. Analysis included interactions between male (also female) parental behaviour (number of visits per hour), territorial behaviour (numbers of chases and songs per hour), condition (tarsus:weight ratio) and the proportion of the nest sired through EPP. Definitions of the above behaviours and their occurrence are covered in more detail in

Chapter three. Condition was measured as the ratio of tarsus length (mm) to mass (g) to indicate the weight of the bird in relation to its size (i.e.: big and fat versus big and skinny). Details of methods for measurements are specified in Chapter two. Territorial encroachments of male and female Bellbirds were analysed with the proportion of young resultant from EPP or intraspecific brood parasitism.

Differences between nests in the proportion of young sired through EPP were tested using the Kruskal-Wallis test. Differences in bandsharing values between females and social mates versus females and genetic mates were tested using t tests.

4.4. Results

4.4.1. Bandsharing patterns

Banding patterns differed for each individual for each probe used. The mean number of bands per individual was 30.56 (± 4.0 SE) for probe 33.15; 21.23 (± 1.3 SE) for probe CA; and 22.7 (± 1.5 SE) for the two probes combined.

Background bandsharing was relatively high between adults with 0.58 (± 0.04SE), 0.52 (± 0.02 SE) and 0.50 (±0.03SE) for probes 33.15, CA and both combined, respectively. The typical background bandsharing for outbred populations is 0.2-0.3 (e.g.: Arden, Ma, Ewen, Armstrong & Lambert, 1997; Orell, Rytkonen, Launonen, Welling, Kumpulainen & Bachman, 1997). Due to the lower bandsharing produced by combining the two probes, only results from the combination of the two probes will be presented for bandsharing values.

The mean ± S.E bandsharing between chicks and males assigned as genetic fathers was 0.485 (±0.02). This value was similar to the mean bandsharing between chicks and mothers which was 0.50 (±0.03 SE). Offspring shared significantly more bands with males assigned as genetic fathers through absence of novel bands, than those of non-fathers (presumptive unrelated males) ($t=3.06$; $df = 49$; $p<0.01$). Similarly, offspring had significantly higher bandsharing among mothers (three cases with irregularity excluded, see section 4.4.3) than non-mothers (presumptive unrelated females) ($t=1.76$, $df = 47$, $p<0.05$). A comparison of bandsharing coefficients and novel bands for chicks and putative fathers showed a significant negative correlation ($r_s=-0.51$, $n=24$, $P=0.01$; Figure 4.2.A), which indicated that the more novel bands present in the fingerprint, the less is shared between offspring and males. A similar trend was found for chick-mother bandsharing coefficients, although the relationship was statistically insignificant ($r_s=-0.314$, $n=24$, $P=0.13$; Figure 4.2.B). This provides justification for paternity assignment or exclusion based on the number of novel fragments detected since the number of novel fragments is supported here by the general levels of bandsharing between chicks and social fathers.

Adult bandsharing values are presented in Table 4.1. Background bandsharing for the 'Bush 1' population of Bellbirds on Tiritiri Matangi Island was of the order of magnitude expected for first order relatives (Jeffreys *et al.*, 1985). The level of bandsharing between males was higher than the bandsharing between neighbouring females, although this was not statistically significant ($t=1.94$, $df =11$, $p=0.07$).

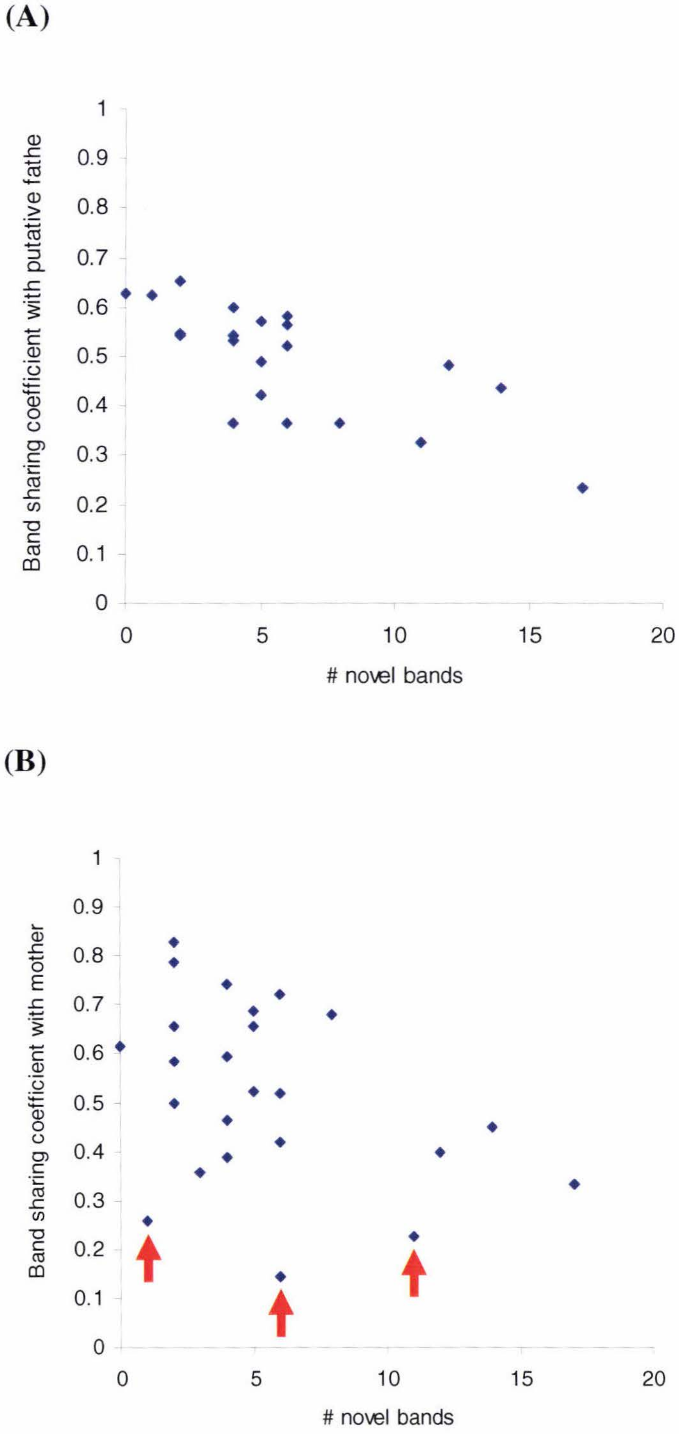


Figure 4.2: Relationship between the band-sharing coefficient and number of novel bands for each nestling compared with (A) the putative father and (B) the mother. The three offspring indicated with red arrows are discussed further in Section 4.4.3.

Table 4.1: Bandsharing coefficients for adults within the Bush 1 Tiritiri Matangi Island Bellbird population. Values are given from the combination of probe 33.15 and probe CA for presumptive unrelated individuals.

	Background	Male:Male	Female:Female	Male:Female	Social Mates	Female: EPM
Mean	0.50	0.55	0.43	0.47	0.47	0.49
±S.E.	±0.03	±0.02	±0.05	±0.01	±0.03	±0.02
n	95	42	9	44	14	11

The band patterns of nestlings consisting of one novel band were assumed to represent a single mutation event. The expected frequency of single mutation events for humans (Jeffreys *et al.*, 1985) and other species (Burke & Bruford, 1987) falls within the range of 10^{-3}. Single novel bands were detected at a rate of 1/110 (~9 x10^{-3}), within the range expected. The pattern of novel bands showed a Poisson distribution for probes 33.15 and CA, but a bimodal distribution for the two probes combined (See Figure 4.3). A bimodal distribution is expected when both mutations and miss-assigned parentage (due to EPP) has occurred (Westneat, 1990).

4.4.2. Paternity Assignment

Twenty-four nestlings from 14 different nests were analysed for paternity. Of these, seven nests were complete and the remaining were partial brood samples. Eight chicks were assigned to their genetic father identified on the gel. In all other cases (n=16) the genetic father was not present on the gel. However, this information was useful in determining the number of chicks not attributable to the social father (present on the gel) and therefore concluded as a result of EPP.

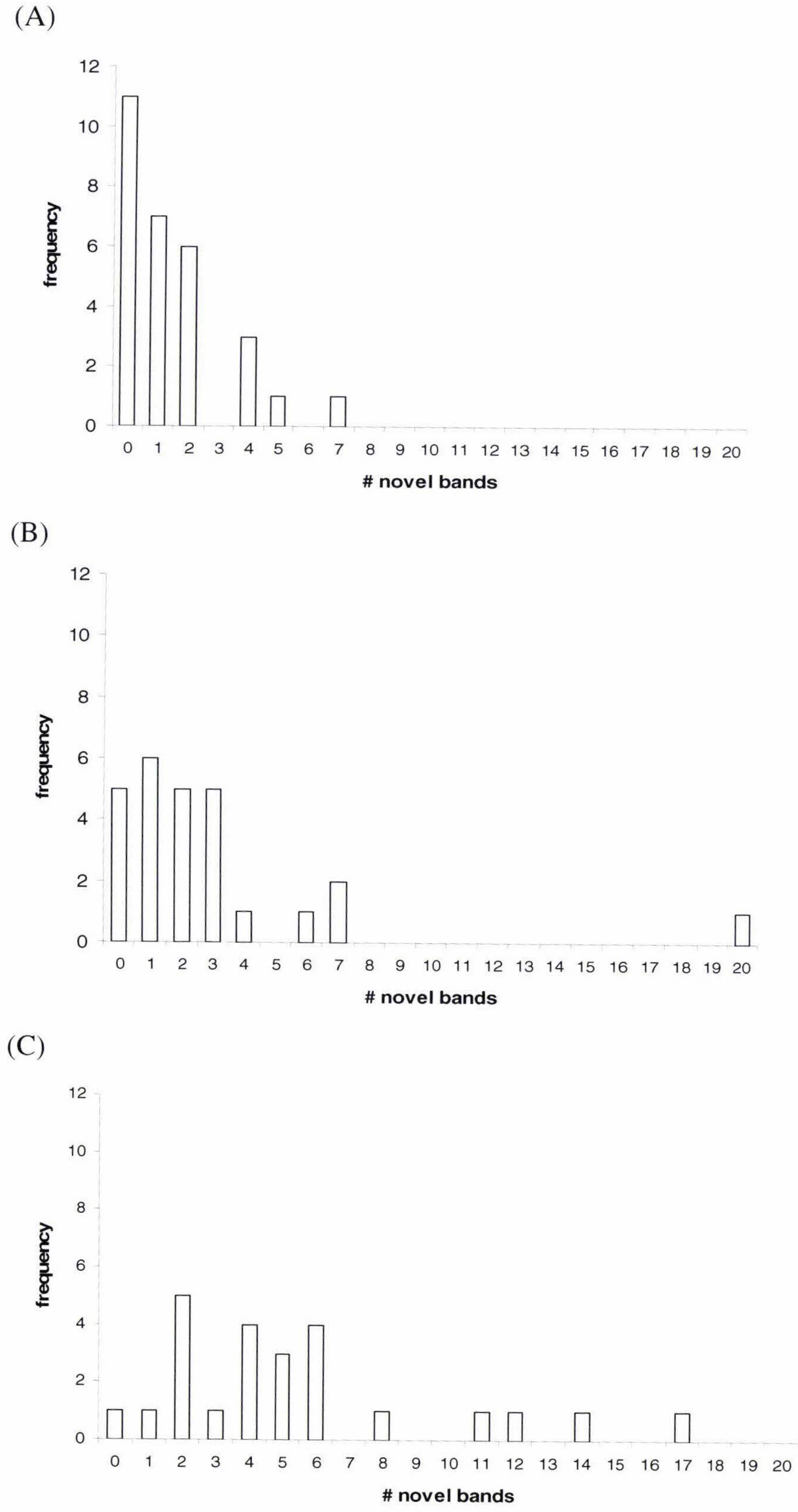


Figure 4.3: Frequency distributions of novel bands present in offspring for (A) Jeffreys 33.15 probe; (B) CA probe and (C) both probes combined.

Twelve (92%) of the thirteen nests analysed for paternity showed at least one incident of EPP or intraspecific brood parasitism. There were three offspring that were a product of genetic monogamy. A nest comprising of one nestling (and one embryo that could not be analysed) showed 100% genetic monogamy, this value may indeed have been different had the embryonic tissue produced a scorable fingerprint.

Of the seven complete clutches examined, the levels of EPP were either 67% EPP (2 of 3 chicks; n=2) or 100% (clutch size ranging from 2-3 chicks; n=5). There were 16 nestlings spread across the seven completely sampled nests, of which 81% (13 /16) of offspring were not attributable to the social father and were therefore a result of EPP. In offspring that were a result of EPP and where the genetic father was able to be identified (n=8), all were the progeny of the male neighbour.

Where the genetic father was identified on the gel, estimates of false paternity assignment ranged from 0 to 10^{-7} (with one case of 0.44), well within the typical range of 10^{-2} (Westneat, 1990). Therefore, it is feasible that the resulting EPP patterns found are a result of true EPP and not false exclusion of parents.

4.4.3. Special Cases

There were three cases of irregularities concerning bandsharing values between the chick and social mother. In all cases the pattern of low bandsharing (<0.30) between offspring and mother and higher bandsharing (>0.40) between offspring and neighbouring females were consistent throughout the different probes used (33.15, CA and combined). These cases were investigated in detail, to determine the combinations of potential parents and offspring and the resulting number of novel fragments produced. In nest B1F2/06, three offspring were produced. One of these nestlings was the result of EPF (Chick 128) due to the presence of six novel fragments and high levels of bandsharing with the putative mother (D= 0.58). However, the other two nestlings (Chick 129 and 130) showed high bandsharing with the social father (D= 0.59 and 0.62, respectively) and remarkably low bandsharing with the social mother (D=0.145 for chick 129 and D=0.259 for chick 130). The neighbouring female, who was laying eggs at the same time as B1F2 female, had relatively high bandsharing with these offspring (D= 0.48 for chick 129 and D=0.38 for chick 130). When evaluated for novel fragments based on the fathers band pattern, the genetic mother of Chick 130 was found to be the social mother (despite low bandsharing), and the maternity of the second chick (Chick 129) remained unknown. It is possible that both the chicks are a result of EPP in which the genetic father was not on the gel. However, this case is ambiguous in that neither EPP nor intraspecific brood parasitism can be rejected. In this case, the chicks with lower bandsharing with the mother had high bandsharing with each other (D=0.69) and low bandsharing with the third (maternally confirmed) chick (D=0.3).

In another nest, TB-1/06, three offspring were produced of which two died due to nest construction failure. The remaining offspring had 21 bands identifiable on the gel, however, it had low bandsharing coefficients with both its social mother (D=0.22) and social father (D=0.35). It is clear that the offspring was a product of either EPP or intraspecific brood parasitism. The TB-1/06 territory overlapped significantly with two females, one of which was laying at the same time as the TB-1 female; unfortunately these individuals were run with their families on separate gels so potential parasitic females could not be detected.

There was one documented case of polygamy in this population and a potential case of polyandry (two males frequented and guarded the nest throughout nesting, however only one fed the nestlings). These two occurred at the same nest in which both chicks present were a result of EPP that was not attributable to either male. However, the polygynous male was confirmed as siring young in a third neighbouring females' nest without showing any sign of guarding or attendance of that nest.

Proportions of EPY differed between nests (Kruskal-Wallis χ^2=15, df = 7, p=0.036). In the males that were identified as genetic fathers of the young, reproductive success seemed to vary between individuals. Social fathers that were not the genetic sires of the progeny they attended usually had genetic offspring in neighbouring nests. This indicates that male and female Bellbirds potentially undertake a mixed mating strategy in that both males and females engage in EPCs.

4.4.4. Correlations with paternity

Parental care in male Bellbirds was not affected by the level of EPP. The number of visits per hour was not significantly correlated with the proportion of young fathered through EPP (r_s=0.179, p=0.179). Similarly the rate of nest attendance of females was not affected by the proportion of EPP in her brood (r_s=0.251, p=0.45). However, territorial behaviour of males was associated with the proportion of EPP. The number of aggressive chases per hour was significantly negatively correlated with the proportion of EPP (r_s=-0.626, n=11, p=0.04) however the number of songs per hour was positively correlated with the proportion of EPP (r_s=0.67, n=11, p=0.04). In these cases, there is a trend for decreased levels of EPP in broods when males chase more frequently rather than sing more frequently. However, there was a non-significant trend of decreasing levels of EPP with increasing male encroachments (r_s=-0.39, p=0.23). It is possible that this is a reflection of observation bias in that not all encroachments within a territory were witnessed.

Territory defence by females, as measured by the number of songs per hour was not correlated with the level of EPP in the nest (r_s=0.3, p=0.29), however, the rate of female singing was positively correlated with the number of female encroachments into the territory (see Chapter three). This suggests that female song does not play an obvious role in attracting extra pair males.

Male condition was not correlated with the amount of cuckoldry suffered (r_s=0.06, p=0.88), however, there was a trend for increased levels of EPP with increasing female condition (r_s=0.69, n=6, *P*=0.12). Finally, bandsharing between females and social mates versus females and genetic mates was not significantly different (t=-1.61, df = 18, p=0.12).

4.5. Discussion

Bellbirds engage in extra pair mating, the level of which varies among individuals. This study presents the first evidence of EPP in Bellbird clutches. The high frequency of EPY in Bellbird nests (92% of nests contained at least one EPY and 81% of offspring were a result of EPP) suggests that their socially monogamous mating system (Heather & Robertson, 1996; Anderson & Craig, 2003) is not indicative of the reproductive strategies employed by males and females. The level of EPY found in nests is higher than that found in Hihi (35%), a species considered highly promiscuous (Ewen *et al.*, 1999). The high level of EPY in broods is comparable to that found for the Superb Fairy

Wren, *Malurus cyaneus*, 76% (Mulder, Dunn, Cockburn, Lazenby-Cohen & Howell, 1994). The level of EPP in Bellbird broods detected in this study (81%) presents some of the highest values found for EPP in passerine birds (see Birkhead & Moller, 1992) for a summary).

Of a small sample of complete clutches, EPF were responsible for a large proportion (67% - 100%) of the young produced. This high level of EPP indicates that males vary greatly in their realized reproductive success (Hasselquist, Bensch & Vonschantz, 1995). In Red-Winged Blackbirds (*Agelaius phoeniceus*), males siring EPY can increase their reproductive success up to 20% (Gibbs *et al.*, 1990). Variation in reproductive success resulting from these mixed reproductive strategies likely contributes to the strong sexual selection pressure evident in Bellbirds (as evidenced by their sexual dimorphism, dichromatism and intense intra-sexual aggression during breeding).

Bandsharing values for this population were reasonably high, and were similar to those found for Blue Duck (0.36-0.51) and the Motuara island population of South Island Robin (0.53) (Lambert & Millar, 1995). The high level of background bandsharing between presumptive unrelated individuals indicates that there is a high level of homozygosity in this population (McRae & Burke, 1996). High levels of homozygosity frequently occur in populations that are inbred (Reeve, Westneat, Noon, Sherman & Aquardo, 1990).There is evidence that the population of Bellbirds on Tiritiri Matangi Island went through a severe bottleneck when the population consisted of 40 individuals in the 1970's (Rimmer, 2004). Extensive research has deducted that levels of bandsharing above 0.51 indicate inbreeding (Papangelou, Harn & Miyamoto, 1998).

Levels of background bandsharing for species that are present on Tiritiri and have also undergone population bottlenecks include 0.20 for the North Island Robin (Arden *et al.*, 1997) and 0.47 for the Hihi (Ewen *et al.*, 1999).

The trend of higher bandsharing between adult neighbouring males suggests that a) there is philopatry of males to their natal territory and/or b) females suffer higher mortality than males causing a greater influx of females into an area. Evidence supporting male philopatry is that sons of dominant territory holding males are higher in status than older males from other areas, suggesting that kinship or hatching site is more important than age in Bellbird male hierarchies (Craig, 1985). However, a long-term population monitoring study of Bellbird on the Poor Knights Islands (*A. melanura oneho*) has shown that individual females had significantly lower probabilities of recapture between years suggesting adult female mortality is higher than that suffered by males (Sagar & Scofield, 2006).To determine whether the high bandsharing between neighbouring males compared to females is a product of natal philopatry or due to higher female mortality will require a longitudinal study of a banded population and is beyond the scope of this research.

Despite the high level of background bandsharing, offspring are expected to share 0.5 + x/2 (where x is the level of bandsharing between presumptive unrelated adults) with their genetic parents (Jeffreys *et al.*, 1985b). The detection of low levels of bandsharing between nestlings and the social mother in the three cases presented above warrants further investigation into the behaviour of females and the possibility of intraspecific brood parasitism.

Care needs to be taken when identifying intraspecific brood parasitism based on genetic evidence alone since errors associated with sizing DNA bands can lead to the false rejection of a female as the genetic mother (Griffith, Lyon & Montgomerie, 2004). As such, behavioural data is needed to support genetic evidence before concluding that intraspecific brood parasitism has occurred (Latif, Grenier, Heath, Ballard & Hauber, 2006). The specific aggression of female Bellbirds towards neighbouring females suggests that further research into this area is needed. Evidence from song playbacks suggests that females could be 'eavesdropping' on each other (Brunton *et al.*, *in review*). A study on intraspecific brood parasitism in Moorhens (Gallinula *chloropus*) suggests that familiarity with neighbouring territories facilitates parasitic laying (McRae & Burke, 1996). The three potential cases of intraspecific brood parasitism presented in this study indicate that this may be an effective strategy employed by female Bellbirds to increase their reproductive success. It should be notes that parasitized nests generally suffer a lower reproductive success due to an increased mortality of parasitic chicks from inconsistent incubation and hatching patterns (Yom-Tov, 1980). Therefore, future analysis of embryonic tissue should be given precedence in order to determine the paternity and maternity of eggs that failed to hatch.

There is a possibility that mismatching bands were a product of incomplete restriction digest (see Birkhead *et al.*, 1990). In order to exclude this possibility, samples including large numbers of novel bands should be re-digested. Although this was not possible for this study, evidence for correct assignment/exclusion of paternity is evident. The possibility that the genetic father was not included on the family gels is extremely high considering the density of the Bellbird population and the fact that these honeyeaters gather within close proximity of conspecifics at nectar resources.

Mating systems are determined by the realised reproductive success of males and females (Orians, 1969). A benefit to males in achieving EPFs is obvious in that the numbers of offspring he produces is increased. However, the benefits to females are less understood and are currently explored through a range of hypotheses. Females benefit from EPCs through the gain of a) fertility insurance (Sheldon, 1994); b) material benefits (including food, courtship feeding and potentially resource access) and c) genetic benefits for offspring (Birkhead & Moller, 1992). Genetic benefits of EPCs include increasing the genetic diversity of a brood (Williams, 1975) and increasing the genetic quality of a brood (the sexy son hypothesis: Fisher, 1930; and the increased disease resistance hypothesis: Zahavi, 1975). Genetic similarity between parents is associated with fitness costs such as hatching failure (Bensch, Hasselquist & Von Schantz, 1994). Increasing the genetic diversity of broods in an inbred population may serve to increase the future reproductive success of offspring. However, there was no pattern found for female Bellbirds obtaining genetically different extra-pair mates when compared to bandsharing within a social pair. These results are similar to patterns found in Coal Tits, *Parus ater* (Schmoll, Quellmalz, Dietrich, Winkel, Epplen & Lubjuhn, 2005) and Great Reed Warblers, *Acrocephalus arundinaceus*, (Hansson, Hasselquist & Bensch, 2004) where extra pair mating was not associated with inbreeding avoidance.

There was no evidence of male condition influencing EPY in nests suggesting that the condition (or quality) of a social male does not relate to the likelihood of being cuckolded. However, the rate that males chased intruders from his territory was associated with an increased proportion of young he sired. There is potential that the ability of a male to defend a territory is a measure of 'quality' in this species. Another measure of quality may be health status; the high prevalence of avian malaria

Plasmodium found in this species (Barraclough *et al.*, *in review*) could be influencing the genetic mate choice of individuals. It would be interesting to investigate the level of parasite infection on mate choice in this population.

Male parental care is expected to vary with increasing uncertainty of paternity (Trivers, 1972). Evidence of this has been shown in the Hihi, where forced copulations of females by EPM result in the social male reducing parental investment due to increased uncertainty of paternity (Ewen & Armstrong, 2000). A review by Moller and Birkhead (1993) has shown that this pattern is consistent in passerine birds. Variation in paternal care is therefore expected to vary greatly considering the low numbers of offspring sired by the social male. However, there was no relationship found between the level of nest attendance (measured in visits per hour) by male Bellbirds and the proportion of broods sired by EPP. Given the high occurrence of EPP across Bellbird nests, it is possible that all males are feeding at a reduced rate due to paternity uncertainty.

Factors that may influence the level of EPP in a population include; the density of a population, breeding synchrony (Stutchbury & Morton, 1995), the sex ratio of an adult population (Ewen *et al.*, 1999), the age of individuals (Bollinger & Gavin, 1991) and supplementary feeding of females (Hoi-Leitner, Hoi, Romero-Pujante & Valera, 1999). The large number of banded birds in this study forms a basis from which the adult sex ratio and age of individuals can be determined in future studies. However, to address the potential affect of supplementary feeding on the mating system of this population, the breeding behaviour of populations that are not supplementary fed should be investigated.

4.6. Conclusion

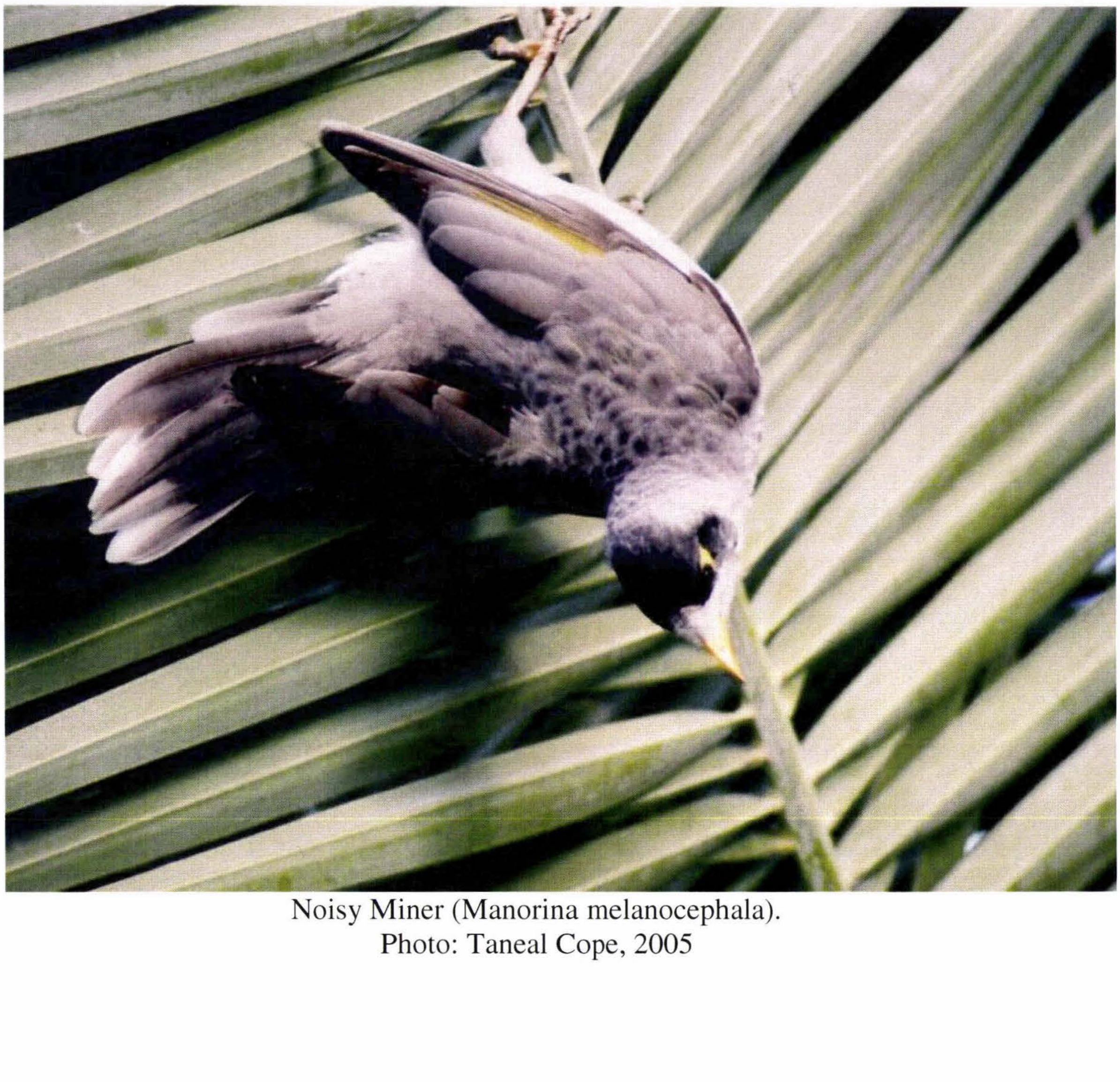
Bellbirds exhibit many interesting factors in their reproductive biology. They are socially monogamous (Anderson & Craig, 2003) yet polygamy and polyandry have been observed (this study). The extremely high rate of extra-pair paternity found in this population of Bellbirds (81%) is unusual compared to the rates found in most passerines (see Birkhead & Moller, 1992) and is more similar to the levels found in the Superb Fairy Wren (*Malurus cyaneus*) (75%), which has been documented as the least faithful bird studied to date (Double, Dawson, Burke & Cockburn, 1997). This result indicates the importance of studying the genetic mating system of a species and also places the Bellbirds' mating strategies as one of the most extreme examples discovered to date.

The behaviours employed by males to prevent cuckoldry include mate guarding and chasing territory intruders. The rate of male chases was associated with decreased levels of extra-pair young in the nest. Females have also been documented as showing aggressive territorial behaviours, but most specifically to other females (Brunton *et al.*, *in review*). The results from this study indicate three possible cases of intraspecific brood parasitism, which may account for female aggression toward other females, in particular neighbouring females.

The population of Bellbirds within the study area on Tiritiri had high levels of background bandsharing between supposedly unrelated neighbours. This finding suggests that either natal philopatry occurs, increasing the relatedness of individuals within an area, or that Bellbirds exhibit close relatedness due to the population bottleneck that occurred on this island. Despite the high bandsharing between individuals, population numbers and individual reproductive success are not low.

CHAPTER FIVE: Conclusions

The mating system of Bellbirds in the context of the Australian honeyeaters.



Noisy Miner (Manorina melanocephala).
Photo: Taneal Cope, 2005

5.1. Outline of this Chapter:

In this chapter, the findings from this research as well as data presented to the 2005 ASSAB conference are discussed in the context of current knowledge of the Australian Meliphagidae. Comparisons are made between physical aspects of Bellbird breeding and those of Australian honeyeater species. Likewise, the social behaviour of the Bellbird is discussed in the context of Australian honeyeaters. The value of Bellbirds as a model species for the exploration of breeding ecology and mating systems is then considered. Finally, management recommendations are presented, and future directions for research are suggested.

5.2. Meliphagidae phylogeny and background

The Superfamily Meliphagoidea consists of three families; the Maluridae (the Fairy Wrens, Emu-Wrens and Grasswrens), Pardalotidae-Acanthizidae (the Australian Warblers and allies) and Meliphagidae (honeyeaters) (Sibley & Monroe, 1990). The term 'honeyeater' is frequently applied a species or genus from the family Meliphagidae and will therefore be used as such throughout this chapter.

The Meliphagidae family consists of 182 species from 42 genera (Driskell & Christidis, 2003). The Australian Chats are excluded from this analysis due to their remarkable adaptations to arid environments and physiological distinction from 'typical' honeyeaters such as the Bellbird. The family Meliphagidae originated and radiated from the Australo-Papuan region (Sibley & Ahlquist, 1985) and occur throughout this region, parts of Indonesia and the islands of the South Pacific (Keast, 1985). Meliphagids are one of the most widespread and diverse groups of birds in Australia (Longmore, 1991). However, many representatives occur within Papua New Guinea and throughout the

Pacific Islands (Keast, 1985), including two species in New Zealand (Driskell *et al.*, 2007; Keast, 1985).

Body size in the meliphagids is diverse, more so than other families of nectivores, e.g.: Nectariniidae and Drepanididae, (Keast, 1985). Three distinctive size classes exist including the small (wing length range of 48-70mm), medium (wing length range of 75-105) and large (wing length range of 114-185mm) sized groups (Keast, 1985). The honeyeaters are characterised by their primarily nectivorous diet and brush-tipped tongue (Keast, 1985; Longmore, 1991). This adaptation means that they play an important role in pollination of flowers in Australia (Paton & Ford, 1977) and New Zealand (Ladley & Kelly, 1996).

New Zealand has three species that were historically defined as Meliphagids; the Tui, Bellbird and Hihi. Recent molecular analysis has resulted in the Hihi being excluded from the Meliphagidae (Driskell, 2001) and considered the sole representative of its own family, Notiomystidae (Driskell *et al.*, 2007), closely related to the New Zealand Wattlebirds, Callaeidae (Ewen, Flux & Ericson, 2006; Driskell *et al.*, 2007). The unusual mating behaviours, nesting habits and physiology of the Hihi suggest that the unusual characteristics shared with the Callaeidae may not be coincidental (Driskell *et al.*, 2007).

Current Phylogenetic analysis of the New Zealand honeyeaters concludes that the Tui and Bellbird are sister taxa (Driskell *et al.*, 2007) within the meliphagid clade. Interestingly, the New Zealand honeyeaters have been estimated to have diverged from the Australo-Papuan honeyeaters approximately 23.8mya, whereas the divergence between the Tui and Bellbird is estimated at 2.9mya (Driskell *et al.*, 2007). However the closest relatives of the New Zealand taxa are yet to be distinguished (Driskell & Christidis, 2003; Driskell *et al.*, 2007). Bellbirds differ from their sister taxa, the Tui, in size (Craig, 1985) and by having sexual dichromatism (Heather & Robertson, 1996).

5.3. Behavioural similarities of the New Zealand Bellbird and the Australian Honeyeaters*

The breeding biology data on each individual species was extracted from (Higgins & Steele, 2001) and cross referenced with available published data. This provided the framework for a comparative analysis of the New Zealand Bellbird and the Australian honeyeaters.

The aim of this exercise was to explore how the mating habits and breeding ecology of Bellbirds, identified in this study, relate to Australian representatives of the Meliphagidae. It was also expected that predictions regarding behaviour for species within the Meliphagidae may be deduced, at least in part, from the similarity of their physical breeding parameters. General methodology for this study is presented in Appendix III.

A Principal Components Analysis (PCA) was conducted using the parameters listed in Appendix III to determine the most similar species to the Bellbird. The resulting PCA that resolved most of the variation was based on bird size, egg size and nest size against incubation time (decreasing), nest height and fledging time (Figure 5.1). The PCA

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* Presented at the 2005 ASSAB Conference, Macquarie University, Sydney.

grouped the Bellbird within the medium sized honeyeaters and was most similar to *Xanthomyza* (*Xanthomyza phyrygia*), the Regent Honeyeater. The Regent Honeyeater is an endangered species (Pizzey & Knight, 2003) that exhibits dominance hierarchies relating to access to nectar sources and is in general decline in Australia due to habitat clearance (Franklin, Menkhorst & Robinson, 1989). It is similar to the Bellbird in physical breeding parameters including incubation length (14 days), time to fledging (16 days) and is considered socially monogamous with pairs that both feed and defend nestlings (Higgins & Steele, 2001). Information on this species is limited and the social and genetic mating systems have not been investigated. However, both the Regent honeyeater and the Bellbird show sexual dimorphism.

5.4. Similarity between characteristic behaviours of the Bellbird and Australian honeyeaters.

Bellbirds represent a typical meliphagid in their behaviours. That is, they are nectivorous, highly territorial and exhibit dominance hierarchies. Bellbirds fall within the approximately 20% of honeyeaters that have been reported to be territorial (Pyke *et al.*, 1996). However, this 20% may indeed be an underestimate considering the large number of species that have not been studied. Aggressive interactions are common within and between the territorial honeyeater species (Armstrong, 1996; Franklin *et al.*, 1989) and usually result in the exclusive use of a territory (Dow, 1976), although not necessarily exclusive access to nectar resources (Stewart & Craig, 1985).

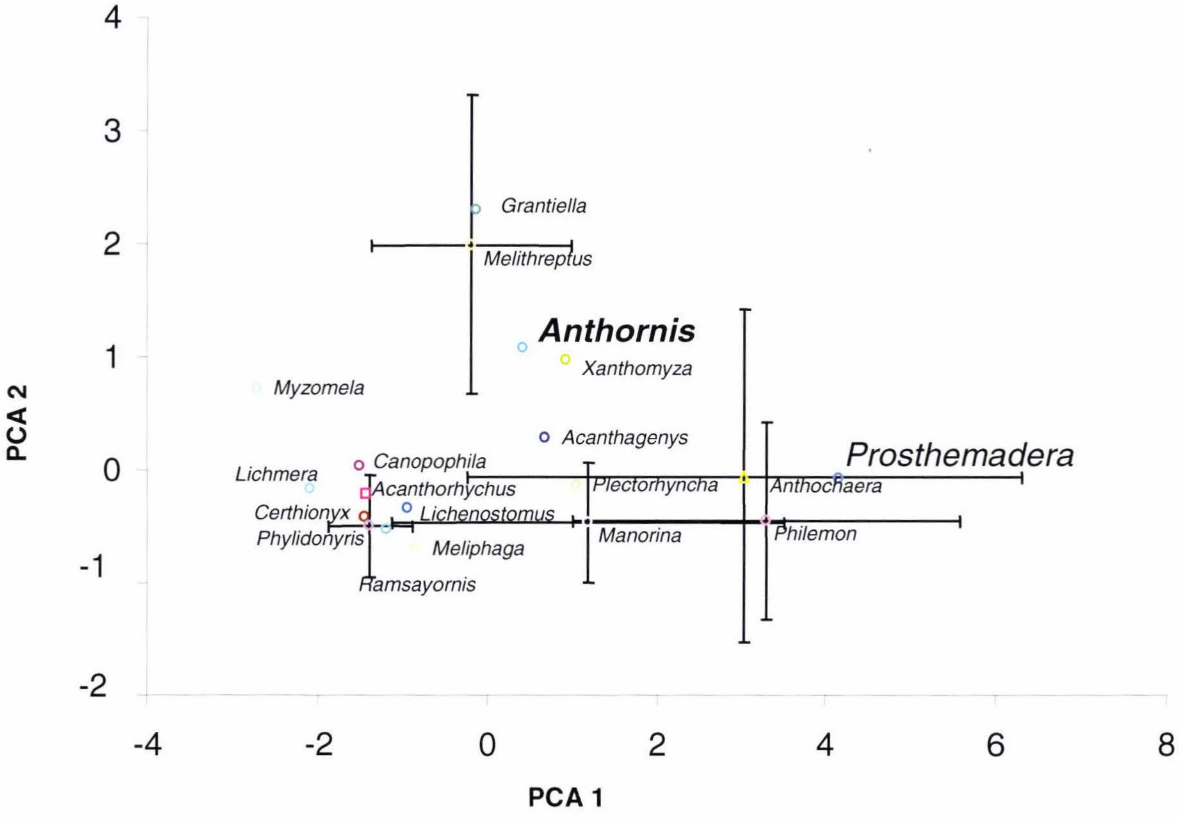

Figure 5. 1: Principal components analysis of physical breeding parameters of the Australian and New Zealand Meliphagidae. Each genus is represented once and bars indicate 95% Confidence Intervals for that genus. Bellbirds are represented as *Anthornis*. Eigen Values for each resultant component are represented on each axis. PCA 1 relates to increasing mass, egg size and nest diameter. PCA 2 relates to decreasing incubation length, increasing nest height and time to fledging.

According to the available literature, Bellbirds are unusual among meliphagids (and passerines) in that females regularly use a large repertoire of song during the winter as well as in the breeding season (Brunton & Li, 2006). Interestingly, results from a recent study suggest that female Bellbirds focus aggression on neighbouring females (Brunton *et al.*, *in review*). This could be explained by the potential intraspecific brood parasitism discussed in Chapter 3, but further investigation into this occurrence is needed.

Since there is only a limited number of published studies on breeding biology of the Australian honeyeaters and because relatively little is known regarding territorial behaviour in females, particularly the use of song, other species may still prove to show a similar behaviour.

Fifty of the 72 species (69%) of the Australian honeyeaters exhibit biparental care. Eleven species show cooperative care (15.3%) and eleven species (15.3%) have no data available. Within the species showing biparental care, visits to the nest were made at a rate comparable to that of Bellbirds; between nine and 14 visits to the nest per hour (e.g.: Helmeted Honeyeaters *Lichenostomus melanops cassidix*: Franklin, Smales, Miller & Menkhorst, 1995; and the Crescent Honeyeater, *Phylidonyris pyrroptera*: Clarke & Clarke, 1999). A possible reason for this common range in visitation rates could be due to energetic constraints of foraging. For instance, Bellbirds have a high energy requirement for basal metabolic rate (Castro & Robertson, 1997). Therefore, if the majority of meliphagids have a similarly high energy requirement, a considerable portion of a parents foraging time would have to be allocated to sustaining itself. Thereby limiting the number of times they can return to provision nestlings.

This argument does not take the influence of variable quality of food resources in account. High quality resources may be expected to reduce the amount of foraging time necessary for parents to meet their own energy requirements. It is known that Bellbirds are regularly displaced from the large, profitable nectar sources (e.g.: Kowhai, *Sophora spp.*) by the more dominant Tui (Craig, 1985) resulting in the need to utilise small flowers, with low profitability (Castro & Robertson, 1997). This may influence the feeding rate of young through resource availability. Individual territories may differ in the quality of resources. Unfortunately, food sources with each territory were unable to be analysed for this study and neither were the size and quality of food items brought to the nest. Therefore, it is unknown how the quality of these may have differed between pairs. More research is needed to understand Bellbird visitation rate and how this may interact with resource quality as well as chick survivorship and growth.

Nestling attendance is higher in females than of males in the Crescent Honeyeater, Helmeted Honeyeater (Franklin *et al.*, 1995) and in Bellbirds. The behaviour of Bellbird females is interesting, as females show a greater level of variation between individuals in nest attendance than males. Variation in attentiveness by males is usually postulated as uncertainty of paternity, variation in individual condition or mating tactics, e.g.: polygyny (Moller & Cuervo, 2000). The variation in females could be explained by female condition, age or resource availability within her territory. Future research should include continued study of this large banded population to determine relative age of individuals, any changes in condition or reproductive success over time and measure resource availability within territories to determine the reasons for variation in nest attendance.

A high occurrence of non-territorial individuals ('strangers') visiting nests has been reported for the Brown-backed Honeyeater, *Ramsayornis modestus*, (Maher, 1988), Crescent Honeyeater (Clarke & Clarke, 1999) and the Noisy Miner, *Manorina melanocephala*, (Whitmore, 1986). This behaviour was observed relatively often in Bellbirds in this study. The above studies, including the present study, all report on the territorial pair aggressively chasing the intruder from the nest. The outcome of nest visits by 'strangers' resulted in infanticide in the Crescent honeyeaters and Noisy

Miners. No cases of infanticide were recorded by visiting Bellbirds to nests of other pairs. One case involved a visiting male carrying food to the incubating female; another case involved a visiting male checking on the nest contents after a disturbance at the nest, meanwhile the remaining six cases of stranger visits involved the male sitting on the rim of the nest looking at the contents.

The majority of honeyeaters have been reported as socially monogamous although there have been a range of mating behaviours observed. Thirty-eight species (54.2%) are recorded as being socially monogamous or consisting of simple pairs, 18 species (25.7%) have shown some level of cooperative breeding, 1 species (1.43%) has been recorded as polygynous and 13 species (18.57%) are data deficient. Out of season copulations have been documented in the Bellbird (Craig, 1985), the Helmeted Honeyeater (Franklin *et al.*, 1995) and has also been recorded in Hummingbirds (Wolf, 1975). In each case, this behaviour was considered to be related to resource access. The rate of occurrence of this behaviour throughout the year and throughout the breeding season would be interesting to investigate and relate to the proportion of extra pair young sired by dominant resource-holding males.

Aggregations of male honeyeaters undertaking displays, to which females are often attracted has been reported in many honeyeaters, including the Bellbird.
'Corroboree' behaviour involves the close proximity of two or more individuals (usually males) that undertake repeated calling, posturing and distinctive movements (Pyke & O'Connor, 1989; see Figure 5.2). It has been recorded in the New Holland Honeyeaters (*Phylidonyris novaehollandiae*), White-cheeked Honeyeaters (*Phylidonyris nigra*) (Pyke & O'Connor, 1989; Armstrong, 1996), Yellow-tufted

Honeyeaters, *Lichenostomus melanops*, (Wakefield, 1958) and Noisy Miners (Dow, 1975). Functions for this behaviour have been suggested as strengthening or establishing breeding ranges (Pyke & O'Connor, 1989) and asserting dominance (Craig & Douglas, 1986). Further investigation into the occurrence, context, initiation and outcome of this behaviour would help to improve knowledge of male-male interactions and the role of this behaviour in Bellbird social systems.

Figure 5. 2: Male Bellbird posturing and displaying in a 'coroboree' aggregation. Photo: Peter Reese, 2005.

5.5. Sexual dimorphism and mating systems in the Meliphagidae.

Based on Darwin's theory of sexual selection (Darwin, 1871), sexual dimorphism is predicted to result as a consequence of high levels of male-male competition for mating partners. In mating systems where male competition for females is reduced (e.g.: monogamy), the pressures from sexual selection are expected to be less significant, leading to fewer sexually dimorphic traits. The majority of species (85.7%, n=70) in the Meliphagidae show very little or no sexual dimorphism. However, 10 species show sexual dimorphism relating to size and/or plumage, ranging in variation between species. Bellbirds exhibit sexual dimorphism and dichromatism. Sexes can be identified based on large differences in both size measurements and plumage characteristics. The majority of honeyeaters are classified as socially monogamous and the majority (85.7%, n=70) show no distinctive sexual dimorphism. This seems to be a specific feature of the Meliphagidae as other nectivorous birds are known to show high levels of sexual dimorphism (e.g.: the Hummingbirds, most of which exhibit highly promiscuous mating systems (Wolf & Stiles, 1970). Based on the high level of sexual dimorphism apparent in Bellbirds, one could speculate that they should exhibit a promiscuous mating system, polygynandry, similar to Hihi (Figure 2).

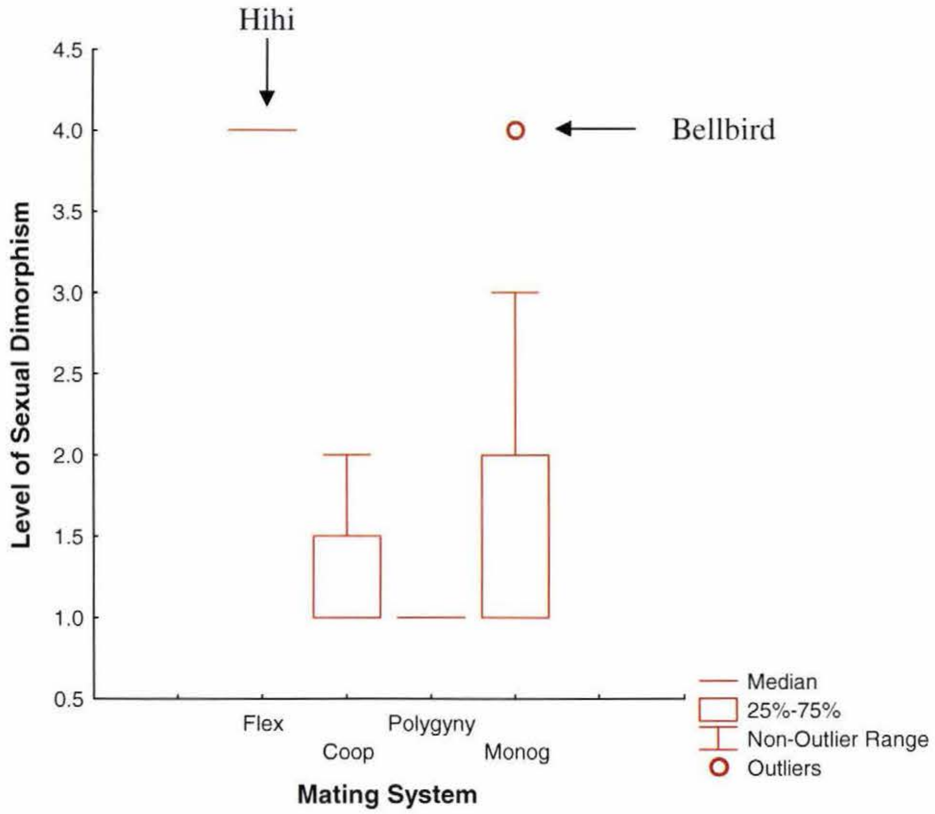

Figure 5.3: Distribution of current mating system classification and level of sexual dimorphism for the Australian and New Zealand honeyeaters. Mating systems include Polygynandry (Flex) of the Hihi, for comparison; Cooperative breeders (Coop), Polygyny and Social Monogamy (Monog). Sexual dimorphism is represented as additive levels of size dimorphism (1-2) and dichromatism (1-2), totalling a maximum of 4.

5.6. The genetic mating system of the Bellbird in relation to current knowledge of the Australian honeyeaters.

Within the honeyeater species studied, there has been a large diversity of breeding behaviours reported including cooperative breeding, social monogamy and seasonal polygyny. A large portion of honeyeaters are cooperative breeders (Clarke, 1995). Cooperative breeding is defined as the presence of non-breeding adults assisting in the nesting attempts of breeding adults (Ligon, 1999). In addition to this, colonial nesting in the Regent Honeyeater (Geering & French, 1998) and New Holland Honeyeater (McFarland, 1986) has been observed. Social monogamy has been recorded in a large proportion of honeyeaters, although most are lacking published confirmation. Of the published studies available, the Yellow-faced Honeyeater, *Lichenostomus chrysops*, (Clarke, Schipper, Boulton, & Ewen, 2003) and Crescent Honeyeater (Clarke & Clarke, 1999) have shown social monogamy both within and between seasons. Seasonal polygyny has been recorded in the Rufous-banded Honeyeater, *Conopophila albogularis* (Noske, 1998), where pairs were polygynous in one season and returned to monogamy the following season.

To date, only three species of Australian honeyeaters have had their mating systems analysed through molecular ecology. These involved sexually monomorphic, cooperative breeders (Clarke & Clarke, 1999). Molecular analysis has indicated genetic monogamy in the Bell Miner (Conrad, Clarke, Robertson & Boag, 1998), Noisy Miner (Poldmaa, Montgomerie & Boag, 1995) and New Holland Honeyeater (unpub.data, *in* Clarke & Clarke, 1999).

This study presents the first analysis of molecular paternity in a sexually dimorphic, socially monogamous Meliphagid. The Bellbird is the first species within the Meliphagidae family to show high levels of extra-pair paternity (81% EPP) associated with sexual dimorphism and dichromatism. In addition, levels of EPP in this island population are comparable to the rate found in what has been described by (Double *et al.*, 1997) as the "least faithful" passerine, the Superb Fairy-Wren *Malurus cyaneus* (a small, sexually dimorphic and dichromatic cooperatively breeding bird, that is also a member of the superfamily Meliphagoidea). The Superb Fairy-Wren demonstrated EPP in 76% of offspring (Mulder *et al.*, 1994).

5.7. The New Zealand Bellbird as a model species for understanding breeding biology and mating systems.

The relatively widespread distribution of Bellbirds on the mainland suggests that this species is capable of surviving in reasonable numbers in the presence of introduced mammalian predators. However, the population of Bellbirds on Tiritiri is typical of offshore islands which are the closest representative of pre-colonisation New Zealand bird densities (Diamond & Veitch, 1981). The counts of Bellbirds on Tiritiri has increased from 24 individuals to numbers estimated over 500 (Veitch *et al.*, *pers.comm*). This high density, closed island population means that large numbers of individuals can be caught and banded and are reasonable easy to study.

The complex social interactions including dominance hierarchies (Craig & Douglas, 1986), female-female aggression through song (Brunton *et al.*, *in review*), mate guarding, pair bonding and biparental care present an interesting framework for understanding tactics employed by each sex to increase their reproductive output. The marked sexual dimorphism between males and females in size and plumage suggests that sexual selection is acting within this species. Individual Bellbirds vary in size, reproductive success, song (Li, 2002), access to resources (Craig & Douglas, 1986) and health (Barraclough *et al.*, *in review*). Thereby providing an opportunity to study these factors and the role they play in the breeding biology and mating tactics of this species.

This study found typical behaviours representative of social monogamy including single male-female pair bonds and biparental care. However, the results from this research indicate that Bellbirds exhibit extreme levels of promiscuity equivalent to the highest level of EPP recorded in passerines to date. This indicates the importance of genetic analysis of paternity in sexually dimorphic birds for further understanding of sexual selection. In addition, the unusual feature of female-female aggression through song leads to speculation over the extent of female intrasexual competition for male mates. The possible cases of intraspecific brood parasitism presented in this study leads to further questions on the reproductive tactics employed by females in this high density population.

The results from this research contribute significantly to improving the knowledge of an endemic New Zealand bird species as well as adding to the existing knowledge of the Meliphagidae family and general passerine behaviour. However, further understanding of male and female Bellbird behaviours would contribute significantly to research on behavioural ecology.

5.8. Directions for future research:

Possible directions for future research, stemming from the findings and observations of this study, are listed below:

- Resource defence experiments (increasing and depleting resources to determine levels of territoriality, resource access and resource defence).
- Relate parental investment to territory quality, measured by the energy production of natural food sources within a territory.
- Comparison of larger sample sizes of paternal investment in nests with extra pair young and those indicating genetic monogamy.
- Detainment experiments of males within the female's fertile period to examine the effect of certainty of paternity on male parental investment.
- Intrusion experiments including male and female playbacks throughout the year and within breeding seasons as well the placement of mounted specimens near the nest to determine extent of reactions from male and female territory owners.
- Experimental placement of eggs in nests of laying birds to determine the reaction of female Bellbirds to possible intraspecific brood parasitism.
- Video recording of Bellbird nests during the laying stage to determine laying patterns and identify possible intraspecific brood parasitism.
- Recording and playback experiments of male aggregation behaviours (corroboree) to determine how they are initiated, the outcome of each aggregation and the reaction of other male and female Bellbirds within the area.
- Analysis of female reproductive organs to determine the presence or absence of sperm storage tubules.
- The influence of population sex ratio on the breeding system of Bellbirds.

5.9. Management recommendations:

- Banding of Bellbirds should be exercised with caution. Due to band injuries associated with the sizes of split bands, all future banding of females should be undertaken with ‘b’ sized colour and metal bands. Nestlings should have their sex determined in the nest before banding so as to apply the correct size band to each sex.

- Molecular analysis determined that levels of bandsharing within this population were high, similar to those of Hihi on Tiritiri (Ewen *et al.*, 1999), the Motuara Island population of South Island Robin *Petroica australis australis* and within populations of the endangered Blue Duck, *Hymenolaimus malacorhynchos* (Lambert & Millar, 1995). Comparisons to other island populations of Bellbirds (e.g.: Hauturu and the Poor Knights Islands) should be undertaken to determine the extent of the genetic bottleneck within the Tiritiri population. Potential translocations of Bellbirds from Tiritiri should be augmented with individuals from other island sources to increase genetic diversity. However, this would be reliant on the prevalence of Avian Malaria (*Plasmodium*) found within other Bellbird populations.

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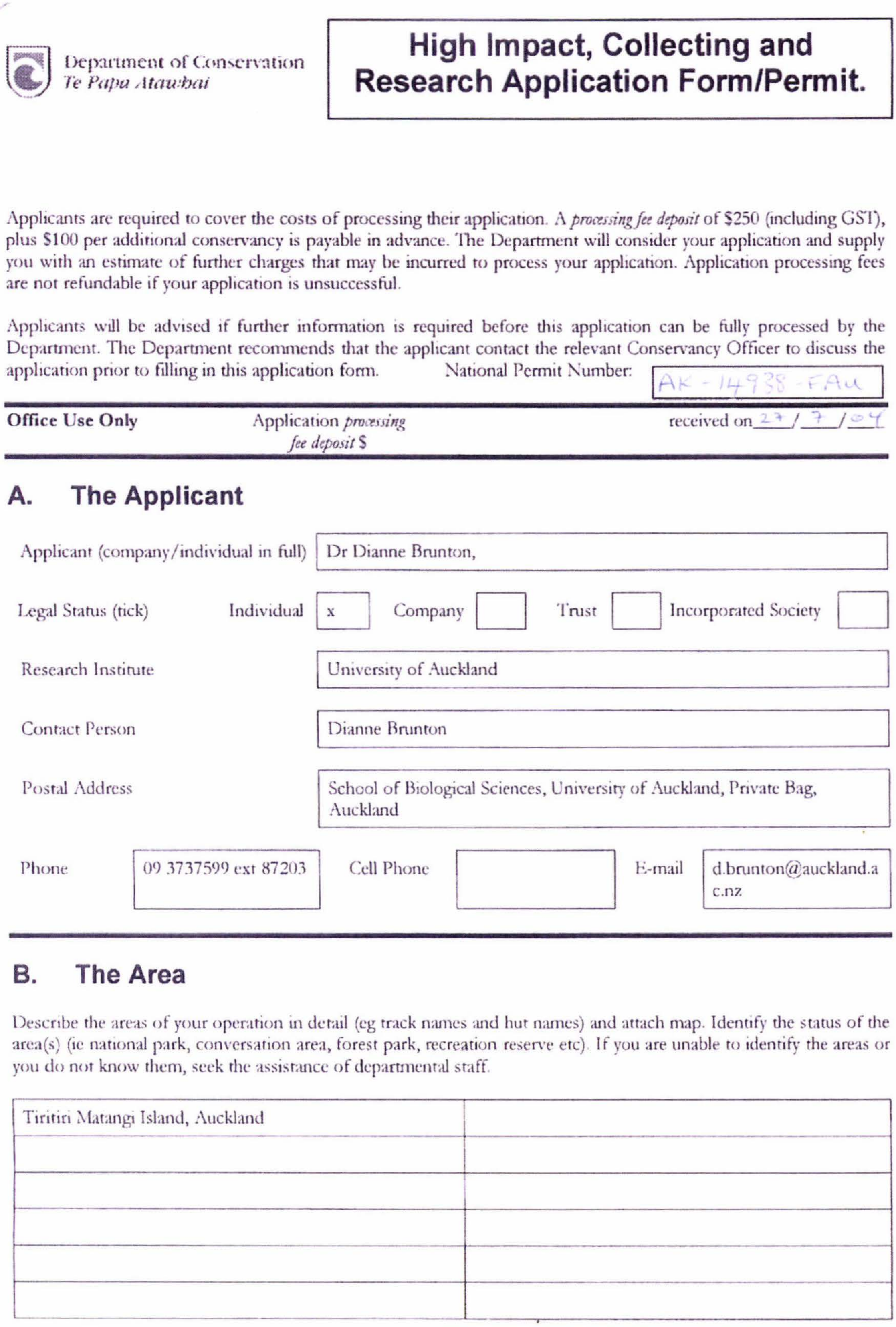
APPENDIX I



Department of Conservation
Te Papa Atawhai

High Impact, Collecting and Research Application Form/Permit.

Applicants are required to cover the costs of processing their application. A *processing fee deposit* of $250 (including GST), plus $100 per additional conservancy is payable in advance. The Department will consider your application and supply you with an estimate of further charges that may be incurred to process your application. Application processing fees are not refundable if your application is unsuccessful.

Applicants will be advised if further information is required before this application can be fully processed by the Department. The Department recommends that the applicant contact the relevant Conservancy Officer to discuss the application prior to filling in this application form. National Permit Number: AK - 14938 - FAu

Office Use Only Application *processing fee deposit* $ received on 27 / 7 / 04

A. The Applicant

Applicant (company/individual in full): Dr Dianne Brunton,

Legal Status (tick): Individual ☒ Company ☐ Trust ☐ Incorporated Society ☐

Research Institute: University of Auckland

Contact Person: Dianne Brunton

Postal Address: School of Biological Sciences, University of Auckland, Private Bag, Auckland

Phone: 09 3737599 ext 87203 Cell Phone: E-mail: d.brunton@auckland.ac.nz

B. The Area

Describe the areas of your operation in detail (eg track names and hut names) and attach map. Identify the status of the area(s) (ie national park, conversation area, forest park, recreation reserve etc). If you are unable to identify the areas or you do not know them, seek the assistance of departmental staff.

Tiritiri Matangi Island, Auckland	

Department of Conservation
Te Papa Atawhai

Our ref: SCR-017

5 August, 2004

Dianne Brunton
School of Biological Sciences
University of Auckland
Private Bag
Auckland

Dear Dianne,

I am writing to inform you that your request to extend your research on Tiritiri Matangi Island on bellbirds has been granted. The research permit that was issued to you in 2001 expired in September 2004. This permit has been renewed and will now expire on the 28th February 2007.

All terms and conditions of the original permit described above are still valid and must be adhered to in addition to the following:

1. The study must be carried out as carefully as possible but if significant adverse effects are observed the study must be stopped immediately and the Programme Manager Biodiversity, Islands in the Warkworth Area Office contacted immediately.
2. The permit holder must follow procedures that are advised by Department of Conservation Programme Managers, to prevent the introduction of disease, rodents, insect or weed species to the sites listed in Schedule 1. The Permittee will ensure that all field equipment is washed clean and sterilised with anti viral solutions prior to entering sites. Equipment must also be sealed in containers so both the Permittee and DOC can be certain it is free of rodents and invertebrates. Footwear and clothing must be free of mud and seeds.
3. The Permit Holder(s) must liaise with and follow advice given by resident Department of Conservation Rangers.
4. The Permit Holder(s) must be clearly identifiable off-track while on Tiritiri Matangi Island and must wear the name-tag provided by Resident Department of Conservation Rangers.
5. The Permittee must not impact on any other absolutely protected wildlife, or other research or management activities at a site.

Bunkhouse accommodation must be booked well in advance through Department of Conservation staff on the island.

Yours sincerely

Rolien Elliot
Area Manager
Warkworth Area Office
For Auckland Conservator

Warkworth Area Office
28 Baxter Street, PO Box 474, Warkworth, New Zealand
Telephone 09-425 7812, Fax 09 425 7813

APPENDIX II

DNA Extraction, Precipitation and Purification

100 µl of blood/buffer mixture was added to 300 µl of SET extraction buffer (10mM Tris-HCl; pH 8.0; 50mM $NaCl_2$; 10mM EDTA) and 20 µl of proteinase K (10mg/ml; stored at -20C) in a fresh Eppendorf tube. The Proteinase K/blood solution was incubated overnight with rotation at 55°C. The solution was then heated to 95°C for 10minutes to deactivate the proteinase K. 400 µl of buffer-saturated phenol (stored at 4°C) was added to the solution (in a fume hood) to denature and remove lipids and degraded proteins. The solution was mixed by gentle rocking for 30minutes at 20rpm before it was centrifuged at 13000rpm for 5minutes.

The phenol was removed by carefully pipetting the lower layer and saved until the DNA extraction had been checked. 400 µl of Phenol: Chloroform: isoamyl alcohol (at a ratio of 25:24:1) was then added to remove any remaining lipids and proteins. The solution was mixed and centrifuged as for the phenol extraction. 400 µl of Chloroform: isoamyl alcohol (24:1) was then added to remove any remaining phenol. The solution was rocked and centrifuged as before. The lower layer of chloroform was then carefully pipetted from the DNA extract and discarded.

40 µl of 3M NaOAc (pH 5.2) was added to the DNA extract with 800 µl of 100% ethanol (chilled to -20°C) and the solution was mixed well by inversion and then centrifuged at 13000rpm for 10minutes. The supernatant was carefully removed without disturbing the DNA pellet that had formed at the base of the Eppendorf tube. The DNA pellet was washed twice with 700 µl of 70% ethanol (chilled to -20°C) and then centrifuged at 13000rpm for 10minutes. The product was dried under vacuum at 30°C

for 2minutes and then re-suspended in 50-100 μl of TE buffer (pH 8.0; 10mM Tris-HCl, 0.5mM EDTA) dependent on the size of the DNA pellet. The solution was rotated overnight at 4°C and stored at the same temperature.DNA concentration was measured using a Nanodrop (ND1000 v3.1.2).

Genetic Sexing

In birds, the heterogametic sex is the female (which harbours the sex chromosomes Z and W) and the homogametic sex is the male (which contains two copies of the Z sex chromosomes). Genetic sexing relies on the specific amplification of regions on the Z and W chromosomes to distinguish between males and females (Griffiths *et al.*, 1998). A highly conserved region of the W and Z chromosome is the CHD (Chromo-Helicase-DNA- binding) gene (Griffiths *et al.*, 1998). Primers to a part of this region; P2 (5'-TCTGCATCGCTAAATCCTTT-3') and P8 (5'-CTCCCAAGGATGAGRAAYTG-3') , developed by Griffiths *et al.*, 1996) amplify different sized DNA fragments of the CHD-W and CHD-Z genes which can be separated by gel electrophoresis. However, as the difference between the fragments is often small a subsequent digestion of the Z-linked *Hae*III restriction enzyme site is required for discrimination. A second set of primers 2718R (5'-ATTGAAATGATCCAGTGCTTG-3') and 2550F (5'-GTTACTGATTCGTCTACGAGA-3'), (developed by Fridolfsson.& Ellegren, 1999) amplify regions of the Z and W CHD locus that are easily differentiated by gel electrophoresis and preclude the need for restriction enzyme digestion.. In this study, both sets of primers were used to a) determine the accuracy of the separate primer sets for bellbird sexing and b) as a reference to ensure accuracy of the results.

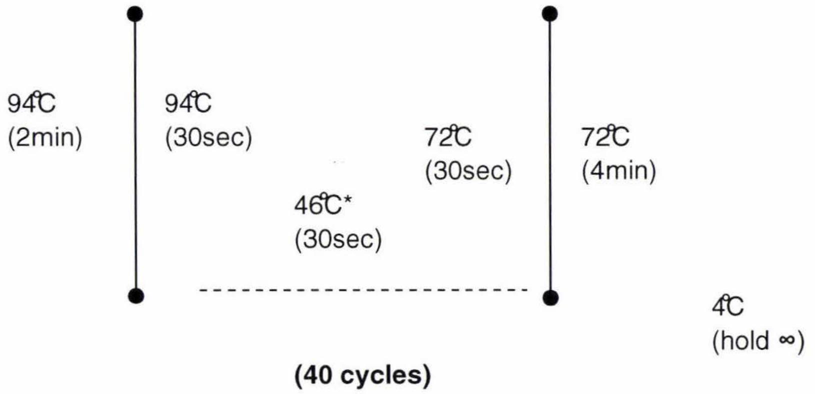
The Polymerase Chain Reaction

Amplification of avian DNA samples was carried out using the Polymerase Chain Reaction (PCR) with the following solution adapted from the Allan Wilson Centres' Avian Sexing Protocols (Anderson, J.M., 2005) :

	Single Sample/Dilution
*PCR Buffer (10x)	1µl
BSA (10mg/ml)	1µl
$MgCl_2$ (50mM)	0.5 µl (2.5mM)
dNTP's (10mM)	0.25 µl (0.25mM)
Primer Forward (10µM)	0.5µl
Primer Reverse (10µM)	0.5µl
Taq (5U)	0.02 µl (0.1U)
DNA	1 µl (1-20ng?)
H_2O	(make solution up to 10µl)

*PCR buffer is 10mM Tris-Cl pH8.3, 50mM Kcl.

Amplification of each 10 µl sample was carried out using a GeneAmp® PCR System 9700 (Applied Biosystems) with the following cycling profile:



The temperature of the primer annealing step was adjusted between 46°C-50°C until optimum DNA amplification was observed. An annealing temperature of 46°C created background and non-specific band amplification, yet higher annealing temperatures (e.g: 50°C) failed to amplify bands. PCR products made using the primers P2/P8 were digested with 1 μl of the restriction enzyme *Hae*III (30min at 37°C) prior to gel electrophoresis. PCR products were electrophoresed in 3% agarose (2% broad standard low melt agarose and 1% wide range standard agarose)in TBE buffer (xxmM Tris base, xxmM Boric acid, xxmM EDTA) at 100V for 20min, stained with ethidium bromide, and then visualized over UV light.

The sizes of the amplification products using the above primers differs between different bird species. For Bellbird we observed the following approximate sizes:

<u>(i) For amplifications using the primer pair P2 and P8</u>

♂ (Z locus) = ~ 300bp

♀ (W locus) = ~ 400bp

<u>(ii) For amplification using the primer pair 2550F and 2718R</u>

♂ (Z locus) = ~ 650bp

♀ (W locus) = ~ 450bp

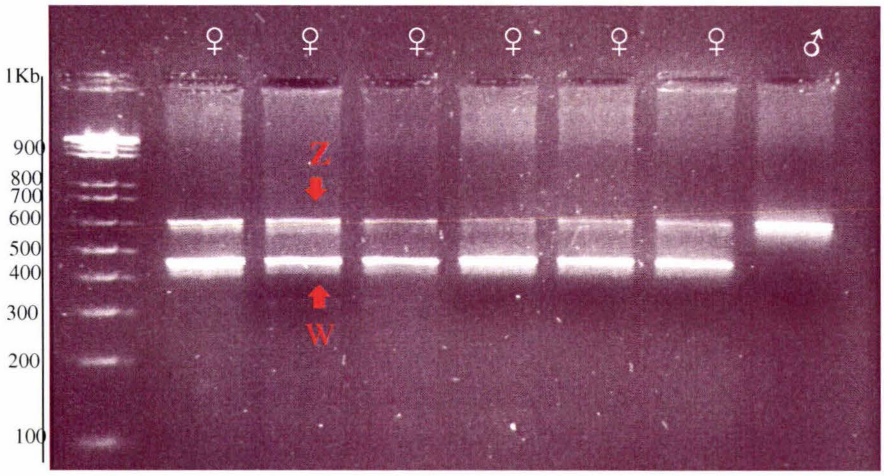


Figure 1: Genetic sexing of Bellbird using primers 2550F and 2718R. The amplified products were separated by electrophoresis in 3% agarose in TBE buffer. DNA fragments amplified from the Z and W chromosome are indicated by the arrows.. Samples 1-6 represent females and sample 7 shows a male. DNA size standards are provided by the 1Kb+ ladder (Gibco-BRL).

Sexes were identified by the presence of two sex loci in females (Z and W) and only one sex locus in males (Z) (See Fig 1).

The 2550F/2718R primer set showed consistently clearer results for Bellbird sexing when compared to the P2/P8 primer set. The P2/P8 primer set showed high levels of background amplification and reference to the 1Kb+ ladder was needed to determine the position of the appropriate sex locus.

APPENDIX III

General methods for the analysis of similarities between the Bellbird and the Australian honeyeaters.

Data collection and analysis

Data on the breeding biology of the Australian and New Zealand honeyeaters were sourced from the Handbook of Australian, New Zealand and Antarctic Birds (Higgins & Steele 2001). In order to determine reliability of the data, cross-references were made to available published accounts (e.g: Geering & French, 1998; Clarke & Clarke, 2000; Clarke, Schipper, Boulton & Ewen, 2003; Franklin *et al.,* 1995; Maher, 1988 and Noske, 1998). Due to insufficient knowledge on species' ecology, data from 30 (of the 72 species) were excluded from analysis. Information was obtained for 42 species representing 19 genera.

Data categories included:

- Average bird size (mass)
- Level of Sexual dimorphism (Minimum 0; Maximum 4)
    - Differences in sizes between males and females were ranked according to differences in: either length or weight (1); and a combination of weight and length (2).
    - Sexual dichromatism was ranked according to differences in: either colour or pattern (1); and a combination of colour and pattern (2)
- Breeding/life history variables
- Nesting characteristics.

Breeding behaviour/ life history variables were ranked into categories. Variables included :

- Mating System:
    1. Flexible (Polygynandry)
    2. Cooperative
    3. Polygamy
    4. Monogamy

- Egg size (length)
- Incubation length
- Clutch size
- Fledging time (length of nestling period)
-

Nesting variables included:

- Nest size (external diameter)
- Nest height (m).

Principal Components Analysis (PCA) was used to determine the factors that represent the most variation between species. Data were analysed using Statistical Analysis Software 9.1 (SAS Institute, 2003).

Results from Principal Components Analysis

The resulting PCA resolved the highest principal components (Prin1 and Prin2) for the factors that explained the most variation. These included bird size (mass), nest size (external diameter), nest height, clutch size, egg size, incubation length and fledging time. The corresponding Eigen values are presented below:

Variable	**Prin1**	**Prin2**
Mass	0.459	-0.116
External Diam	0.439	-0.23
Nest Height	0.347	0.442
Clutch size	0.316	0.262
Egg size	0.45	-0.218
Incub length	0.244	-0.612
Fledg time	0.335	0.495