Evolutionary ecology of multiple ornaments in the golden whistler

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Abstract

The function of multiple display signals in golden whistlers (*Pachycephala pectoralis*) was investigated over three field seasons (2001/02 – 2003/04) at Toolangi State Forest (Victoria, Australia). Male golden whistlers are highly ornamented and possess several elaborate plumage displays, including a yellow breast and a conspicuous white throat patch. In addition, they are highly vociferous and possess large song repertoires.

The study population was located in a high-altitude forest that experiences harsh winter conditions. As a consequence, all individuals migrated from the site annually after the completion of breeding. The short period of favourable climatic conditions appeared to impose significant constraints on breeding biology. The average duration of the breeding season was 2.5 months shorter than the average for other Australian passerines, and each pair attempted to raise only a single brood each year. The short, intense breeding season of golden whistlers at Toolangi is atypical for Australian passerines and shows more similarities to northern hemisphere migratory passerines.

Song is a complex and multifaceted component of the golden whistler’s display repertoire. Male singing rates were low upon arrival at the breeding site but increased dramatically at the onset of breeding and may be used as a paternity guard. Yet, males decreased song rates when high numbers of neighbouring females were fertile, suggesting an inability to simultaneously sing as a paternity guard and pursue extra-pair matings. Song repertoire size was highly variable between males and was partly related to male age. Each repertoire typically consisted of two broad song-types: simple ‘whistle’ songs that may function in long-distance communication and more complex ‘melodic’ songs which were more commonly sung in close-range male encounters. During such encounters, males displayed a hierarchical suite of interactive vocal behaviours, such as song-type matching and song overlapping that may signal male willingness to escalate a contest.

To understand what benefits males may gain from their ornaments, it was important to reliably quantify male reproductive success by assigning paternity to offspring. I developed
six novel DNA microsatellite markers for golden whistlers which allowed paternity assessment with a high degree of certainty (exclusion probability = 0.9997).

Nineteen percent of golden whistler nestlings were sired by extra-pair males. Males with large song repertoires and high quality breast plumage were less likely to be cuckolded, and paradoxically, males singing at high rates during the female fertile period sired fewer young. This pattern may arise because males of different quality adopt alternate signalling strategies. Males that are less likely to attract extra-pair fertilisations may invest more into singing as a within-pair paternity guard. Males singing at high rates also defended larger territories, as did males displaying larger throat patches. Male throat patch size was negatively associated with date of breeding initiation, possibly reflecting earlier arrival of dominant males. Breast colour and song repertoire size seem to be used as cues during female choice at short and long distances, respectively. In contrast, throat patch size and singing rates may function as short- and long-range signals, respectively, during male-male competition.

I investigated experimentally the signalling role of the throat patch via plumage manipulations and field-based simulated territorial intrusion experiments. Females showed no difference in response to the two treatment trials (control vs. reduced throat patches). In contrast, males were slower to respond to rivals with dissimilar throat patches to their own, possibly to prevent costly fighting between highly mismatched individuals, but subsequently displayed lower aggression to all intruders with reduced throat patches. When both throat patch size and singing rates were manipulated, females again ignored variation in patch size, but responded more strongly to high song rate trials. In contrast, males displayed increased aggression towards males with large throat patches and high song rates. Throat patch size may signal intruder status and competitive abilities, while singing rates may reveal current motivation to escalate the contest. The sex-specific differences in responses may reflect differences in the information content of the two signals. Females participate in joint territorial defence and both members of a pair regularly intrude on a neighbouring territory together. Yet disputes between neighbours are sex-specific. Therefore, assessing an intruding male’s throat patch may be unimportant for females. However, females may discern variation in male singing rates if the motivation of both
members of an intruding pair to engage in a contest is correlated. Male singing rates may therefore reliably reflect the female intruder’s motivation during the contest.

In golden whistlers, complex multicomponent displays maximise the information that can be conveyed to conspecifics of both sexes. Each ornament appears to reflect different aspects of male quality and is assessed at different distances.
This is to certify that

(i) the thesis comprises only my original work except where indicated in the preface,
(ii) due acknowledgement has been made in the text to all other material used,
(iii) the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Wouter F.D van Dongen

December 2005
Preface

The research reported in this thesis was conducted after receiving approval from the Faculty of Science Animal Experimentation Ethics Sub-committee at the University of Melbourne (reg. no. 01011). Permission to conduct research on golden whistlers at Toolangi State Forest was issued by Department of Natural Resources and Environment (now Department of Sustainability and Environment; permit number 10001445). All birds were banded under a banding licence issued by the Australian Bird and Bat Banding Scheme (authority number 2478).

In all of the following chapters, I am the principal investigator and primary contributor to all aspects of the work. However, I could not have completed many aspects of my research without the generous assistance of others. Many volunteers worked on this project as field assistants under my supervision. In particular, this involved assisting in the capture and measurement of the birds, searching for nests, and assisting with the simulated territorial intrusion experiments. Most of these tasks could not be carried out alone. Data collected from this field work are presented in Chapters 2, 3, 5, 6 and 7. In addition, several people taught me how to conduct the molecular work necessary for writing Chapters 4 and 5. Everyone who assisted me with the project is listed in the acknowledgments.

This thesis is written as a series of six papers that have been or will be submitted for publication (Chapters 2-7). This approach had lead to some repetition in the method sections of these chapters. Chapter 2 has been published in the *Australian Journal of Zoology* (2005) 53, 213-220 and is co-authored by Larissa Yocom, who was the principal field assistant helping with data collection for this chapter. Chapter 3 has been accepted to be published in *Behaviour*. Chapter 4 has been published in *Molecular Ecology Notes* (2005) 5, 4-6, with my project supervisor, Raoul Mulder, as a co-author. Raoul Mulder will also co-author Chapters 5, 6 and 7. These papers are preceded by an introductory chapter (Chapter 1) and the thesis is concluded with a general discussion (Chapter 8).
Acknowledgements

Since so many people helped with my PhD research, writing these acknowledgments comes with the fear of having left some important people out. Therefore, I’ll do my best to thank all the many people who have contributed in some way to the completion of this thesis, over the four years since I first started this project.

I’d like to thank my supervisor Raoul Mulder for all his intellectual and logistical input and support over the years. Raoul first introduced me to the exciting world of avian sexual selection when he took me on as an honours student six years ago. I’d like to think that I’ve come a long way since those early days and have Raoul to thank for a large part of that development.

Anyone that has helped me with fieldwork knows that working with these birds can often be difficult, frustrating and unrewarding. I sometimes hated those birds! But with so many people willing to lend a hand, it was definitely a fun and memorable experience. Without the help of all my field assistants I would not only have been unable to work with such efficiency and have had so much fun. In particular I thank Larissa Yocom, the best field assistant anyone could ask for. I guess we were both a bit nervous at the beginning about working together as two complete strangers. But I couldn’t have made a more perfect choice and we ended up being an unbeatable duo in the field. You were quick to learn and soon became an invaluable part of the project over the two years that you came to Australia. Plus you still hold the record for the number of olive whistler nests found at Toolangi! But it wasn’t only all that physical slavery that I put you through that I’m grateful for. Thanks also for your intellectual input into successfully running the field seasons and experiments and mostly for all the fun we had out there. However, it wasn’t just a two person show, so I also need to thank Saskia van Dongen (the surprise standout field assistant of the project), Sophie Allebone-Webb, Michelle Simeoni, Kristy van Dongen, Mam, Pap, Grainne Maguire, Robert Ramiarison, Tania Billing, Ellen van Wilgenburg, Emile van Lieshout, Carly Cook, Lindy and Oliver Eyster, Nicole Cranston, Ange Schneider and Meah Velik-Lord.
Fieldwork was only half of the slog I had to get through. When I was done spending countless days out in the bush, I had to drag myself into the lab for months at a time to figure out how promiscuous the little birds really are. I hated it at first, but grew to quite like it and so have to thank Grainne, Patrick-Jean Guay, Greg Adcock, Susan Campbell, Ellen, Tania, Bojun Chiswell, Damian Dowling and Iain Woxvold for all their help and for making it a quite pleasant experience being up there in the lab.

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There’s a wealth of people that contributed in small but important ways throughout the project. Many thanks to the Rushworth banding group for teaching me everything I know about capturing and banding bush birds, in particular, Joan and Charles Sandbrink, Khalid Al-Dabbagh and Aine and Richard Walsh. Thank you to the staff at the Toolangi DSE for logistical support, Ian Gordon and Graham Hepworth for statistical advice (and to you too Grainne, Ellen and Damian), Kees Müller and Brian Elbers for providing cages so I could safely keep the whistlers in captivity during my field experiments, Staffan Andersson for conducting the carotenoid analysis of the whistler feathers, David Paul for assisting in the production of figures for this thesis, David Macmillan who was always there to provide support throughout both my honours and PhD projects, and Raoul, Grainne, Larissa, Amy Rogers, Helen Bishop, Josh van Buskirk, Peter Dunn and Matt Symonds who read through earlier drafts of my chapters. Thanks also to Murray Littlejohn for providing most of the recording and playback equipment I used over the years.

One of the highlights of PhD life is of course the opportunity to hang out with a great bunch of friends, day in and day out. My four and a half years in the department as a PhD student would definitely have been a less exciting experience without all the fun and friendship that came with the tight circle of friends I made, in particular Grainne, Ellen,
Emile, Patrick, Amy, Dave Morton, Damian, Susan, Helen, Verity Bristow, Coralie Reich, Kath McNamara, Tania, Robert, Elisa Bone, Dave Semmens and Ken and Femmie Kraaijeveld.

My parents often complain that they feel underappreciated and never get acknowledged for helping me with all the little things during my PhD, like providing countless (mostly) tasty meals, support and a roof to sleep under. So here’s your own little paragraph of thanks. Thank you for all the little things! Also thanks to Kris and Sas for just generally being a good bunch of sisters.

And that just about sums it up, except to once again thank the most important person of all, Larissa (although haven’t I already thanked you enough in these acknowledgements?). It’s obvious that you were the best field assistant out there, but it was for everything else that I’m most grateful. For flooding me with love, making me laugh, keeping me motivated during the tough times, hours of conversation in person and on the phone, your friendship, all the fun things we got up to and basically making my life endlessly happy over the last three years, regardless of whether we were close together or ridiculously far apart. I’m definitely a better person for having met you.
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Aims

Birds exhibit a large variety of elaborate display signals. These displays vary widely among different species, but typically include visual and acoustic traits such as showy plumage and complex singing behaviour. The functional significance of these elaborate signals, typically more pronounced among males, has attracted considerable attention from researchers in the past few decades. It is now generally accepted that avian display traits advertise signaler quality to conspecifics during male contests over resources or during female mate choice decisions (Andersson 1994). Numerous studies suggest that individuals with more elaborate ornaments are of high genetic or phenotypic quality, enjoy greater reproductive success or have a higher social status. The physiological or social costs associated with signal expression appear to be greater for poor quality individuals (e.g. Hill 1999; Gil and Gahr 2002), discouraging cheating by inferior males and maintaining the reliability of ornament expression as an indicator of male quality.

Theoretical models suggest that individuals should minimise costs associated with the expression of sexual displays by evolving a single, highly informative sexual signal (Pomiankowski and Iwasa 1993; Schluter and Price 1993; Andersson 1994; Iwasa and Pomiankowski 1994; but see Van Doorn and Weissing 2004). However, the relevance of these models has been questioned because in many avian species individuals concurrently display multiple costly display signals.

Møller and Pomiankowski (1993) outlined three broad mechanisms by which multiple sexual displays may be maintained. First, various non-interrelated signals may reflect different aspects of male quality or be directed towards different receivers (the *multiple message* hypothesis). For example, in red-collared widowbirds (*Euplectes ardens*), tail length is under strong female selection, while the size and colour of the plumage collar signals dominance during male contests (Pryke *et al.* 2001). This maximises the efficiency and amount of information that can be conveyed to conspecifics. Alternatively, the
expression of several displays may be inter-correlated and related to a single aspect of 
signaller quality. This not only increases the probability of conspecifics detecting the 
signal, but concurrently reduces the possibility of mistakes being made in assessment of 
signals with high error variances (the back-up message hypothesis). Finally, ornaments 
may be a result of past selection pressure or sensory bias drive, and thus may not currently 
reflect signaller quality (the unreliable message hypothesis). In this case, ornaments only 
persist due to minimal maintenance costs.

A growing body of research has provided some insight into the generality of the three 
proposed mechanisms underlying multicomponent display signals in birds. Multiple 
message signalling appears to be the most common mechanism via which species 
simultaneously express multiple sexual displays (Candolin 2003). Yet surprisingly, despite 
umerous studies on avian multiple ornaments (e.g. northern cardinals, Cardinalis 
cardinalis, Jawor and Breitwisch 2004; satin bowerbirds, Ptilonorhynchus violaceus, 
Doucet and Montgomerie 2003; blue peafowl, Pavo cristatus, Møller and Petrie 2002; 
mallards, Anas platyrhynchos, Omland 1996), very few have concurrently quantified both 
behavioural and plumage ornaments (e.g. Andersson 1989; Møller et al. 1998). This is 
crucial for understanding signal evolution, since the expression of different ornaments is 
typically linked to different aspects of male quality, and thus conspecifics may emphasise 
those ornaments that reveal the most relevant information regarding the receiver. For 
example, in barn swallows, male singing rates reflect parasitic infection rates and immune 
function (Møller 1991a; Saino et al. 1997). Yet, cheaters may also be able to temporarily 
express high singing rates, devaluing the effectiveness of this indicator of male quality. As 
a consequence, females appear to emphasise singing rates in mate choice only when 
expressed in conjunction with costly long tails (Møller et al. 1998). Few studies have also 
investigated different selective pressures imposed on signal expression by male 
competition and female choice (e.g. Pryke et al. 2001) or the opportunity for extra-pair 
matings (e.g. Møller et al. 1998). Although most avian species are socially monogamous, 
males augment their reproductive success to varying degrees by seeking matings with 
additional females (reviewed by Griffith et al. 2002). Females only gain indirect genetic 
benefits from mating with extra-pair males, but typically gain direct benefits, such as high
levels of paternal care, from social mates. Therefore, due to differences in the costs and constraints associated with expression of different signals, the sexual cues used to assess social mate quality may differ from cues used in extra-pair mate choice (e.g. Catchpole 1986; Bensch and Hasselquist 1992). Thus, the role of male ornamentation should ideally be considered both in the context of within- and extra-pair mate choice. Finally, experimental manipulation of multiple ornaments to quantify sex-specific differences in signal perception are crucial to gain insights the function of multicomponent displays, but remain uncommon (e.g. Hagelin and Ligon 2001; Hagelin 2002).

In this thesis I report on an investigation into the function of complex multicomponent displays in the golden whistler (*Pachycephala pectoralis*), in which I attempt to address many of the issues raised above. The golden whistler is a sexually dichromatic species in which males are highly ornamented (with yellow breast plumage, a yellow nape band, a white throat patch, black chin stripe and crown, and olive wings and back) while females are relatively drab, with uniform grey/brown plumage. Males are also highly vociferous and exhibit a high complexity in song structure and singing behaviour. Using a combination of field observations, field-based experiments and laboratory-based paternity testing, I assess the information content of the various signals displayed by golden whistlers, identify the most likely receivers of each signal and ultimately ask why males of this species express multicomponent display signals.

In Chapter 2, I provide a comprehensive overview of the breeding biology of a golden whistler population at the high-altitude study site, Toolangi State Forest, Victoria, Australia. Although the golden whistler is a common and ubiquitous species, a detailed description of its breeding biology was still lacking when I commenced my research. This information is necessary to understand findings described in subsequent chapters. As the study population was migratory, I explored possible constraints on mating strategies by the need to undergo annual migration to and from the breeding grounds.

Song is a conspicuous component of the golden whistler’s signalling strategy. I found that there was considerable temporal and intrapopulation variation in singing behaviour and
song structure in this species, and therefore the amount of information that may be encoded in different aspects of whistler song is potentially large. In Chapter 3 I examine sources of variation in singing behaviour and song complexity of golden whistlers. I describe how whistler song varies between individuals, in different singing contexts and across different breeding stages, and discuss the extent to which male singing strategies are influenced by selection pressures imposed by male competition and female choice.

In order to gain a comprehensive understanding of the signalling properties of each male sexual ornament, it is essential to accurately quantify male within-pair and extra-pair reproductive success. In Chapter 4, I describe the isolation and characterisation of six polymorphic DNA microsatellite markers developed specifically for unambiguous assignment of paternity in the golden whistler.

Chapter 5 investigates the principal question of this thesis: why do male golden whistlers possess multiple display signals? I first describe the genetic mating system of this species using the genotyping techniques described in Chapter 4. I then quantify variation in ornaments and aspects of male quality between males to examine two broad themes: 1) the information content of each signal and 2) the most likely receivers of these ornaments (i.e. males or females). I discuss my findings in relation to the three mechanisms by which multiple signals can be maintained within a species, as outlined by Møller and Pomiankowski (1993).

A key finding in Chapter 5 was that the song output and throat patch of males may function as signal of dominance during male-male contests. I tested this idea experimentally by conducting a number of field-based experiments. I first manipulated the size of the throat patch of caged males, which were then presented to individuals of both sexes in simulated territorial intrusions (Chapter 6). Sex-specific behavioural responses to variation in throat patch size provided further information on the likely receivers of this signal. Furthermore, variation among males in responses to simulated intruders possessing different sized throat patches suggest a mechanism by which honesty in this signal is maintained. Chapter 7 complements the preceding chapter by detailing an additional experiment in which I
concurrently manipulated the size of the throat patch and apparent singing rates of caged males during the simulated intrusions. This experiment allowed the assessment of the relative importance of a static (throat patch) and dynamic (singing rate) trait in signalling male dominance in this species.

I conclude the thesis in Chapter 8 by integrating my empirical findings and discussing the broader implications of this work for the study of avian sexual selection. Finally, I list promising avenues for research which may further extend our knowledge on the complex interactions between display signals in male golden whistlers.
Breeding biology of a migratory Australian passerine, the golden whistler
(*Pachycephala pectoralis*)


Abstract

The breeding biology of Australian passerines is characterised by long breeding seasons and the potential to produce multiple broods within a single season. However, many species undergo a yearly migration from their breeding sites to climatically milder wintering grounds. This migratory behaviour may impose constraints on the breeding biology of these species. I studied a migratory population of golden whistlers (*Pachycephala pectoralis*) at Toolangi State Forest, Victoria. Individuals typically arrived in early September, attempted to produce a single successful brood over a three-month period and departed again in late April. Both site- and pair-fidelity was high, with an average of 66% of males returning to the same site, re-pairing with the same female in up to 100% of cases. Nest depredation was common, with 45% of nesting attempts failing due to depredation. Each pair initiated an average of 1.7 nests per season, resulting in an average of 1.2 fledglings per pair each season. The short breeding season and single-broodedness of the golden whistlers at Toolangi is atypical amongst Australian passerines and suggests constraints may be imposed on species undergoing annual migration. In this regard, golden whistlers show more similarities to northern hemisphere migratory passerines than the sedentary passerines of the southern hemisphere.

Introduction

The annual migration made by many birds from their breeding grounds to climatically milder wintering areas may impose significant constraints on their potential for reproduction. Migratory behaviour may be energetically costly and be associated with increased levels of competition during territory acquisition and relatively short breeding
seasons (Alerstam 1990). In contrast, non-migratory species often benefit from long breeding seasons and the potential for multi-brooding (Woinarski 1985; Rowley and Russell 1991). Furthermore, migration patterns may dictate variance in reproductive success among males, for example if earlier-arriving individuals gain access to superior territories or mates and thus enjoy greater reproductive output than later arrivals (Kokko 1999; Møller et al. 2003; Møller et al. 2004).

Although predictable long-distance migration is common amongst temperate northern hemisphere passerines, it is relatively rare in Australia. This may reflect an adaptation to milder and less variable climatic conditions between seasons (Rowley and Russell 1991) and may have led to smaller clutch sizes, greater longevity, longer breeding seasons and relatively few true migrants in Australia (Woinarski 1985; Rowley and Russell 1991). Yet up to 40% of Australian species exhibit partial migration, where some populations of a species migrate each year, while others remain resident on breeding territories all year round (Chan 2001; Griffioen and Clarke 2002). Partial migration may represent a response to highly variable climatic patterns and resource availability across years, two characteristic features of the Australian landscape. Individuals may make decisions about whether to remain on their breeding territory throughout winter based on trends in the local weather and food abundance (Chan 2001).

Given the high proportion of sedentary species in the southern hemisphere, there is a predictable bias towards the study of resident populations of birds (Clarke 1997). Studies of the behaviour and breeding biology of Australian migratory species, by comparison, are rare (eg. Robinson 1990; Bridges 1994a; Magrath 1999; Clarke et al. 2003). This lack of data hinders comparisons of Australian migrants with resident species and their northern hemisphere migratory counterparts. This is unfortunate, because such comparisons may well be illuminating in identifying factors that have contributed to the evolution of migration in Australia, and that influence the breeding biology of migratory species. This information may furthermore be of value to conservationists: life history traits associated with migratory behaviour, such as variation in habitat use throughout the year, form an essential component of species management models (Clarke 1997).
The family Pachycephalidae is represented by eight species of whistlers in mainland Australia and Tasmania. Populations of these species range from resident, to partially migratory and exclusively migratory (Higgins and Peter 2002). This family thus presents an interesting model for the study of the evolution and maintenance of migratory behaviour. However, to date, only the breeding biology of a migratory population of rufous whistlers (*Pachycephala rufiventris*) has been studied (Bridges 1994a; Bridges 1994b). The golden whistler (*P. pectoralis*) is a partial migrant inhabiting forests throughout southern and eastern Australia. Some populations have been described as sedentary or resident, while others are exclusively migratory (Higgins and Peter 2002). Although the golden whistler is a common and ubiquitous species, most studies have focussed on its foraging behaviour (Bell 1986; MacNally 2000; Craig 2002) and there have been no comprehensive studies of its breeding biology. Here I describe the breeding biology of an exclusively migratory population of golden whistlers in Toolangi State Forest, Victoria. I document temporal patterns of migration in relation to breeding behaviour over three breeding seasons and discuss possible implications of migratory behaviour for the breeding biology of this species.

**Materials and methods**

**Study site**

This study was carried out between September 2001 and February 2004 at Toolangi State Forest, Victoria, Australia (37°31’ S, 145° 32’E). The study area initially covered 80 ha within a continuous stretch of forest covering approximately 38 000 ha (2001), but I expanded this to 106 ha in September 2002. The predominant vegetation consists of a mountain ash (*Eucalyptus regnans*) canopy, with an understorey of soft tree fern (*Dicksonia antarctica*), musk daisy-bush (*Olearia argophylla*) and hazel pomaderris (*Pomaderris aspera*) in the gullies and mountain correia (*Dodonaea angustissima*) and mint balm bush (*Prostanthera melissifolia*) on the drier slopes. The site is 850 m above sea level and experiences high rainfall (mean annual rainfall: 1 377 mm) and a relatively cool climate (coldest month: July – mean minimum temperature = 3.7 °C, mean maximum
temperature = 9.8 °C; hottest month: February – mean minimum temperature = 12.3 °C, mean maximum temperature = 24.8 °C).

**Capture methods and morphometric measures**

Individuals were captured upon arrival at the study site (during the pre-breeding phase) or whilst undergoing breeding activities, across three breeding seasons from 2001/2 to 2003/4. Birds were captured in mist nets using three techniques: playback of male song only, playback of male song with the presentation of a live caged decoy bird, or flushing individuals from the nest during the incubation phase. The most successful technique was capture off the nest (although this had to occur when the eggs were at least five days old, or the parents were likely to abandon). Prior to the commencement of breeding, capture with a live decoy was the most successful technique. Via daily censuses I was confident that I had banded most individuals within the study site and was aware of those individuals that remained unbanded (proportion of banded individuals – 2001: 88% (36/41), 2002: 96% (47/49), 2003: 85% (39/46).

I banded each captured bird with a metal band supplied by the Australian Bird and Bat Banding Scheme and a unique combination of three coloured leg rings. I measured standard biometrics including head-bill length (distance from the tip of the beak to the back of the head, to the nearest 0.1 mm, using dial callipers), tarsus length (nearest 0.1 mm, callipers), flattened wing length (nearest 0.5 mm, blunt-ended ruler) and body mass (nearest 0.1 g, spring balance). I also took a small blood sample (50 µl) from the brachial vein for subsequent genetic analysis. For whistler pairs in which both members possessed female-like plumage (i.e. when the male was still in sub-adult plumage), I determined the identity of the male via behavioural observations. This was later confirmed by sexing using molecular techniques after Griffiths *et al.* (1998).

**Behavioural observations**

The population was monitored daily throughout the breeding season. I monitored 25 pairs in 2001, 27 in 2002 and 24 in 2003. After locating an individual, I noted its behaviour and
position using a set of reference points spread throughout the study site (97 reference points over 106 hectares). Male territories were then mapped using the geographic information system software package ArcView GIS 3.2 (Environmental Systems Research Institute 1999). I mapped territories for males in 2001 and 2002. Territory sizes were calculated where I had a minimum of eight fixes per male from different days (mean number of fixes 2001: 11.1 ± 3.0 SD fixes/male, \( n = 18 \), 2002: 12.4 ± 4.0 SD fixes/male, \( n = 20 \)).

Nests were found by following an individual until the nest was visited. A pair was assumed not to have an active nest if no evidence of nesting behaviour was seen during an uninterrupted hour of observation. Only females were followed if a pair was assumed to be nest building as males do not contribute to this activity. Females built nests by collecting nesting material from vegetation or the ground and making regular trips back to the nest. Both males and females incubated and fed nestlings and males regularly sang when returning to the nest, facilitating nest location. A nesting attempt was defined as the initiation of any nest, regardless of the subsequent fate of that nest.

All located nests were monitored every two or three days. A blood sample was taken from nestlings when they were at least three days old for molecular sexing and paternity analysis. Nestlings were fitted with an ABBBS metal identification band and a unique colour combination of three leg bands when at least seven days old. At this time they were also weighed and length of the both tarsi measured. The mean tarsus length for each individual was used for subsequent analyses. If the age of the nestling was unknown, it was estimated using a range of morphological features, such as body mass, tarsus length, development and emergence of feather shafts and whether the eyes were open. These characteristics were compared to patterns of development observed in nestlings of known age.

I quantified parental effort at the nest by conducting nest watches during incubation throughout 2001. Males usually did not assist in incubation until the eggs were five days old, so all watches occurred after this time. Incubation observations were for a standard 90-minute period, during which time I was hidden from view at a distance of 8 – 12 m from
the nest. During the observation, I recorded the length of each incubation bout for each sex, along with the number of vocalisations.

*Statistical analysis*

All data were tested for normality and transformed where necessary. Parametric tests were then used, except in cases where data transformation did not normalise the data, in which case non-parametric tests were used. Data are presented as means and standard deviations unless otherwise stated.

Although 33 adult males were captured at the study site, an additional 24 individuals were captured between 0.5 and 5 km from the site for a separate study (Chapter 6, 7). When reporting mean morphometric measures of males, I pooled the data of all males captured at all sites ($n = 57$).

Exact arrival dates of males were difficult to determine due to poor weather conditions and their inconspicuousness during this period. Thus, the population arrival date was taken as the mean date on which the first four males of the season were sighted. This also minimised the effects of any uncharacteristically early arrivals: in most years the majority of males were first sighted approximately one week after the first male sighting of the season. Female arrival dates were not quantified in detail.

To investigate patterns of variation in nestling mass, I controlled for within-brood effects by averaging the weight and age of each nestling in the brood. The residuals from a logistic regression of mean nestling weight against mean age were then used for each brood. In this way, relative nestling weight was unaffected by within-brood factors, such as preferential feeding of nestlings and age.

The proportion of time spent incubating by each sex during the 90-minute nest observations was used to represent incubation effort. These data were transformed into arcsine $\sqrt{x}$ values.
before analysis. Climatic data for the Mt St Leonard’s Weather Station in Toolangi State Forest were obtained from the Bureau of Meteorology.

**Results**

*Male plumage development*

Thirty three adult males were banded at the study site. Five of these adults possessed traces of sub-adult plumage, consisting of streaks of grey in the black crown, paler yellow plumage and undefined outer edges of the throat patch. In addition, a further six males were captured in sub-adult plumage (i.e. in female-like plumage), five of which possessed traces of adult plumage. This typically consisted of streaks of yellow in the breast, olive on the back and wings or black throughout the crown. Three sub-adults returned to the site in subsequent years, all in adult plumage. Sub-adult males were presumably in their second year, as all immature individuals resembled females and possessed rufous-coloured wing primary feathers.

*Sex differences in morphometrics*

Males had larger head-bill lengths, longer tails and longer wings than females (Table 1). Morphometric measurements of sub-adult males were similar to those of adult males except for wing length. Mean body mass for individuals of both sexes was 27.5 ± 1.9 g (n = 87).

*Arrival and departure dates*

The location(s) of the wintering grounds for this population is unknown. Males arrived at Toolangi State Forest in early to mid September (population arrival date - 2001: 10 September [range: 4 - 18 September]; 2002: 22 September [range: 17 - 26 September]; 2003: 17 September [range: 3 - 23 September]), with females arriving a week or two later.
Departure from the study site occurred after the cessation of breeding and the completion of the moulting cycle. This occurred in late April in all three years.

*Pairing and territory fidelity*

Both males and females vigorously defended territories throughout the breeding season. Mean territory size was 2.5 ha (range 1.0 – 4.2 ha) and did not vary across years (mean territory size 2001: 2.5 ± 1.2 ha; 2002: 2.5 ± 0.8 ha; F$_{1,34} = 0.013$, p = 0.911). Territory sizes for each male tended to be correlated between years ($r^2 = 0.251$, F$_{1,10} = 3.356$, p = 0.097).

Of 77 individuals captured at the study site, 92% were paired and defended a territory within the site. Five of the remaining six individuals (one sub-adult and four of unknown sex) were presumably floaters or defended territories outside the area I regularly monitored. These individuals were not resighted after their initial capture. The remaining floater was a female that paired with a territory-holding male after the disappearance of his own partner.

Return rates of males were high (2002: 73%, $n = 26$; 2003: 59%, $n = 27$; Fig. 1). Up to 100% of males resighted in subsequent years re-paired with the same female, when that female was known to be alive; the exact figure was difficult to determine because some individuals of both sexes were unbanded (2001: minimum 81%, maximum 100%; 2002: minimum 73%, maximum 100%). Territory fidelity was also high. Over the three years, 34 of 35 males (97%) returned to approximately the same territory. Thirty eight percent (10/26) of males were present in all three seasons. No fledglings were sighted at the site in the years following their natal season.

In most cases, pairs remained together for the entire breeding season. The only exception was the male described above that paired with a floater female after his partner disappeared. I did not observe any divorce between partners within a season where both members of the pair were known to be alive for that entire season. Four of out the five pairs where both members were known to be alive remained together for the entire three-year study period.
Nest site selection

The nest of the golden whistler consisted of a loose cup of small twigs, grass and other vegetation matter bound together with spider web. Placement of the nest was usually in a small fork of a shrub. At Toolangi State Forest, a large number of nests were built in musk daisy bush, although this was not the most common plant species (pers. obs.). One hundred and nine nests were located, of which 42% were built in musk daisy bush, 30% in austral mulberry, mountain correa or soft tree fern and the remaining 28% in various other plant species. Nests were built at an average height of 3.0 ± 1.9 m (range: 1 – 13 m) above the ground.

During nest site selection, both the male and female hopped quietly together through the vegetation. Once a potential site was found, the female commenced mock building of the nest, positioning herself on the nest site and drawing in any nesting material that was within reach. Several potential nest sites were presumably tested in this way before proper nest building commenced.

Nesting characteristics

The breeding season of golden whistlers at Toolangi State Forest typically commenced in late October, with the last nests fledging in February (mean day of first egg 2001: 30 November [range: 11 October – 16 December]; 2002: 15 November [range: 20 October – 2 December]; 2003: 26 November [range: 14 November – 2 December]). Although a small proportion of pairs commenced breeding in October, 89% of nests were initiated in November and December (Fig. 2).

Clutches typically contained two or three eggs, with the largest clutches laid in December ($F_{3,92} = 6.412, p < 0.001$). This difference may possibly be a reflection of variation in clutch size after nest failure: nests of the first attempts of the season for each pair contained two eggs in 88% of cases and three eggs in 12% of cases. However, after nest failure, the proportion of nests containing three eggs rose to 36% (chi-squared test: $\chi^2 = 30.42$, d.f. = 1, $p < 0.001$).
The interval between the laying and hatching of the first egg was 17 – 19 days, with 90% of eggs hatching in 18 days. Both sexes contributed to incubation and nestling provisioning. Females incubated for a greater proportion of the time (females: 53 ± 16%, males: 30 ± 18%, unattended: 17 ± 19%; Mann-Whitney U test: $U = 3.84, n_{\text{males}} = n_{\text{females}} = 26, p < 0.001$). However, the mean bout length of both sexes was similar (females: 25.4 ± 16.5 minutes/bout, males: 18.9 ± 12.9 minutes/bout; $F_{1,48} = 2.47, p = 0.123$). Instead, females performed more incubation bouts during each sampling trial (females: 1.46 ± 0.56 bouts/h, males: 0.78 ± 0.51 bouts/h; Mann-Whitney U test: $U = 3.68, n_{\text{males}} = n_{\text{females}} = 26, p < 0.001$). Male incubation effort was correlated with clutch age. Males incubated older clutches for a greater proportion of time ($r^2 = 0.24, F_{1,23} = 7.62, p = 0.013$). However, this was solely due to males not commencing incubation until the clutch was older than five days. If incubation bouts in which the clutch was less than five days old were removed from the analysis, the relationship disappeared ($r^2 = 0.052, F_{1,17} = 0.924, p = 0.350$). I did not compile detailed observations of parental effort during nestling provisioning.

Individuals advertised their arrival at the nest by singing a simple song, to which the incubating individual often responded. Birds occasionally left the nest upon hearing this characteristic song or, more often, waited until their partner arrived before departing. Females sang 4.2 ± 8.7 songs/h on the nest (range: 0.0 – 39.3 songs/h) and males 1.2 ± 3.9 songs/h (range: 0.0 – 17.1 songs/h). This difference approached significance (Mann-Whitney U test: $U = 1.82, n_{\text{males}} = 20, n_{\text{females}} = 25, p = 0.068$).

Both sexes contributed to nest defence, bombarding potential nest predators (i.e. when I removed nestlings to take measurements), flattening their body dorso-ventrally and emitting a high pitched screeching call until the predator departed. I did not witness any nest predation events.

Nestling sex ratio and development

Fifty percent of the 130 nestlings that were sexed using molecular techniques were male and 50% were female. Relative nestling mass decreased throughout the season ($r^2 = 0.17, \ldots$).
F₁,₂₄ = 5.00, p = 0.035), but was not influenced by the time of day (r² = 0.09, F₁,₂₄ = 2.39, p = 0.135), nor by brood size (F₁,₂₄ = 0.025, p = 0.875). Nestlings typically fledged between 10 and 12 days after hatching. Fledglings initially possessed rich rufous plumage, but all had moulted into immature plumage after eight weeks. Fledglings were capable of full flight about two weeks after leaving the nest and were fed by both parents for at least six weeks after fledging. Each parent typically fed only one fledging.

Factors affecting fledging success

Table 2 outlines the mean output of each pair per nest per season. On average, each pair initiated 1.70 nests per season (range 1 – 4), producing 1.16 fledglings each season. Golden whistlers at Toolangi were single-brooded, and ceased all breeding activities after the first successful brood was produced. However, many nesting pairs failed to produce any fledglings within a given breeding season. Thirty-six percent of nesting attempts successfully produced at least one fledgling, resulting in a mean of 0.68 fledglings being produced per attempt.

Thirteen percent of nests were abandoned either during nest building or incubation. Nests initiated in December had the highest success rate, with 43% of nests producing at least one fledgling. This was followed by November, in which 33% of nests were successful (Fig. 2). Overall, the greatest cause of nest failure was depredation, both during incubation and nestling brooding. Although no predation events were observed, several likely predators were present at the study site, including birds such as the grey currawong (Strepera versicolor) and grey shrike thrush (Colluricincla harmonica), and snakes, most notably the tiger snake (Notechis scutatus). Depredated nests were typically left intact, with rarely any evidence of egg or nestling remains.

The likelihood of a brood fledging successfully seemed to be affected, in part, by the plant species in which the pair nested. Of the four plant species most commonly used as nesting substrate, nests placed in mountain correa had lower success compared to austral mulberry, soft tree fern and balm mint bush (chi-squared test: χ² = 11.05, d.f. = 3, p = 0.011). This was attributed to higher levels of predation and abandonment in this plant species.
However, there was no effect of nest height on fate of nest (i.e. abandoned, predated, fledged: $F_{3,97} = 0.701, p = 0.553$), nor when considering nests as successful or unsuccessful ($F_{1,99} = 0.273, p = 0.603$).

Breeding success was also, in part, explained by the period of time for which a breeding pair had been together (i.e. ‘old pairs’ were partnered with the same individual as in the previous season, while ‘new pairs’ were paired with a new mate). Although there was no difference between the mean date that the two groups commenced breeding (old pairs: 19 November ± 9 days; new pairs: 16 November ± 6 days; $F_{1,15} = 1.77, p = 0.204$), or mean clutch size (old pairs: $2.1 ± 0.3$ eggs/nest; new pairs: $2.1 ± 0.8$ eggs/nest; $F_{1,17} = 0.952, p = 0.343$), old pairs tended to fledge more offspring compared to newly formed pairs (old pairs: $1.6 ± 2.8$ fledglings/season, new pairs: $0.6 ± 2.8$ fledglings/season; $F_{1,16} = 3.95, p = 0.064$).

**Sex-specific behaviours**

Males were on occasion observed to perform a trill display. This involved an individual hopping quietly or remaining stationary, usually in low vegetation and emitting a high pitch trilling sound. No other individuals were ever present during this display. However, the probability of such a display occurring seemed to be dependent on breeding stage. Males always performed the trill display during egg incubation or nestling provisioning. Therefore, this may be a defensive signal against potential predators. In support of this, a male performed the display on one occasion when the nestlings were removed from the nest for measuring.

Males were highly territorial and male-male disputes were common. Countersinging with neighbouring males was commonly observed. If a male intruded on a neighbour’s territory, then characteristic chasing and posturing resulted. Males landed on nearby vegetation, flatted themselves dorso-ventrally, puffed out their throat feathers to display their throat patch and repeatedly sang simple songs.
Females were also frequently involved in these territorial disputes, with the same characteristic chasing, singing and posturing. However, females only ever chased other females.

In comparison to males, females sang relatively short and simple songs (unpublished data). Songs were predominately sung during intrusions by neighbouring individuals and often overlapped or immediately followed male songs.

**Discussion**

Golden whistlers at Toolangi State Forest were obligate migrants that exhibited high site and pair fidelity, with male return rates of 73 and 59% in the two years of the study. They also exhibited a number of breeding characteristics uncommon in the Australian passerine fauna, such as a short breeding season and single-broodedness. These adaptations may be related to the migratory behaviour of the population, although the high altitude of the breeding site may also impose constraints on the whistler’s breeding season.

*Consequences of migratory behaviour for breeding*

In environments where harsh winter conditions are prevalent, migration to wintering grounds with milder climates and higher food abundance may be advantageous (Alerstam 1990). At 850 m above sea level, Toolangi State Forest experiences high precipitation (rain and some snow) and low temperatures during the winter months. Golden whistlers feed on invertebrates on the foliage of eucalypts and shrubs, and the biomass of this prey is reduced during the winter months (Woinarski and Cullen 1984). However, a range of other food sources, such as leaf litter invertebrates, remain common throughout the year and species that utilise these resources, such as the superb lyrebird (*Menura novaehollandiae*) and eastern whipbird (*Psophodes olivaceus*), are more often year-round residents (Ashton 1975). Summer migrants are more common in high altitude habitats such as mountain ash forests, although this may partly be due to increased competition for resources between resident species and migrants throughout the foothills (Loyn 1998). At lower altitudes
within the same forest, winter climates are milder and here golden whistlers are year-round residents (Loyn 1985).

Migratory behaviour appeared to have a possible influence on the breeding strategies of golden whistlers. Breeding did not commence until about 10 weeks after arrival at the site, presumably to await favourable climatic conditions and higher invertebrate abundances for successful breeding. Breeding ceased approximately 12 weeks before departure from the site, after fledglings had reached independence and adults had completed the annual moult. The average duration of the breeding season over the three-year study was 3.1 months, compared to an average of 5.5 months for other Australian passerines (Yom-Tov 1987). In this respect, the short, intense breeding season of the golden whistler more closely resembles that of birds in the northern hemisphere. Although, these patterns may be a direct consequence of the environmental constraints associated with breeding at high altitudes, such as shortened periods of favourable conditions, previous studies have suggested that migratory behaviour alone, without altitudinal effects, may impose constraints on the length of a population’s breeding season. For example, Ford and Trèmont (2000) reported that, in the same high-altitude habitat, a resident population of red wattlebirds (*Anthochaera curunculata*) bred for 5 – 6 months of the year, while migratory noisy friarbirds (*Philemon corniculatus*) finished all breeding activities after 3 – 4 months.

It has been suggested that extended breeding seasons and the potential for multibroodedness that is characteristic of so many Australian species may be an adaptation to high levels of nest predation (Martin 1993). Only 36% of nesting attempts made by the golden whistlers produced independent offspring, a success rate comparable to other Australian open-nesting species (e.g. Dowling 2003; Rogers and Mulder 2004). Although most Australian migratory species probably experience the same high levels of nest depredation, they have a much shorter period in which to produce a successful brood. This may partly explain why species such as the golden whistlers at Toolangi State Forest only raise a single successful brood within a season. Unfortunately, no reliable data are available for average brood numbers within other populations of this species (Higgins and Peter 2002).
High return rates and territory site fidelity seem to be a common phenomenon among *Pachycephala* species. Bridges (1994b) reported similar return rates for the rufous whistler, with 96.3% of males returning to the same territory and 95.8% of pairs reuniting in multiple breeding seasons. It is possible that the return rate of individuals in my study was even higher than reported, if individuals settled on territories nearby, but outside the limits of the study area. Site fidelity may be advantageous for maintaining familiarity with potential nest and foraging sites, and refuges from predators (Greenwood 1980; Greenwood and Harvey 1982). Individuals that have high reproductive success in one year often have a higher probability of returning to the same site in the following year (Paton and Edwards Jr 1996; Haas 1998; Hoover 2003). Site fidelity may also reduce costly disputes between neighbours if individuals are already familiar with surrounding birds (Brindley 1991; Stoddard *et al.* 1991; Burt *et al.* 2001). Migratory species generally exhibit lower natal philopatry than resident species (Weatherhead and Forbes 1994). Natal philopatry seems to be a rare or even non-existent phenomenon in the golden whistler population, although it is possible that I may have detected higher return rates of fledglings had I sampled a larger area and continued this study for a larger number of years. Nevertheless, studies of other southern hemisphere migratory passerines have reported similarly low return rates for fledglings (Bridges 1994a; Magrath 1999; Clarke *et al.* 2003). For example, in migratory rufous whistlers only three of 17 fledglings returned to the study site to breed, at an average distance of 1.4 km from their natal territory (Bridges 1994a).

In contrast to North American migratory passerines, which experience high levels of divorce (Rowley 1983), Australian migrants generally exhibit higher rates of social pair fidelity across breeding seasons (Robinson 1990; Bridges 1994a). Territory fidelity can facilitate re-pairing with the same individual across seasons, if both members of a pair return to the same site year after year. This may increase breeding efficiency by decreasing partner search time, allowing earlier breeding attempts and potentially greater reproductive success (Rowley 1983). In support of this, pairs of golden whistlers that remained with the same individual as in the previous season fledged more offspring in comparison to newly
formed pairs, although this may be confounded by age, as new pairs may be more likely to be composed of younger and less experienced individuals.

In conclusion, golden whistlers at Toolangi exhibit some life-history characteristics that appear to be typical of migratory species, including a reduced breeding season and the production of a single brood per breeding season. This is in contrast to the majority of Australian species, which have long breeding seasons and the potential for multiple broods within the same breeding season. The importance of migration as a life-history trait moulding breeding parameters is suggested by the fact that the breeding biology of this migratory population of golden whistlers shows more similarities with those of northern hemisphere migratory species than sedentary southern hemisphere species.
Table 1. Mean morphometric measurements of male and female golden whistlers at Toolangi State Forest. \( n_m, n_{sm} \) and \( n_f \) are sample sizes for adult and sub-adult males, and females respectively. \( n_m \) includes males captured at the study site and those captured within 5 km from the site for a separate study (Chapter 6, 7). P-values refer to differences between adult males and females. * denotes an additional significant difference between adult and sub-adult males (p < 0.05). Values after means indicate standard errors. Lengths are in millimetres and weight in grams.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Sub-adult males</th>
<th>Females</th>
<th>( n_m )</th>
<th>( n_{sm} )</th>
<th>( n_f )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head-bill length</td>
<td>38.4 ± 0.1</td>
<td>38.4 ± 0.3</td>
<td>38.1 ± 0.1</td>
<td>57</td>
<td>8</td>
<td>37</td>
<td>0.013</td>
</tr>
<tr>
<td>Tarsus length</td>
<td>22.0 ± 0.1</td>
<td>22.0 ± 0.1</td>
<td>22.1 ± 0.1</td>
<td>56</td>
<td>8</td>
<td>36</td>
<td>0.637</td>
</tr>
<tr>
<td>Wing length</td>
<td>99.5 ± 0.2</td>
<td>98.2 ± 0.2</td>
<td>96.2 ± 0.3</td>
<td>56</td>
<td>8</td>
<td>37</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Tail length</td>
<td>80.9 ± 0.3</td>
<td>81.3 ± 0.7</td>
<td>79.6 ± 0.3</td>
<td>56</td>
<td>8</td>
<td>36</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight</td>
<td>27.5 ± 0.3</td>
<td>27.4 ± 0.6</td>
<td>27.6 ± 0.4</td>
<td>44</td>
<td>7</td>
<td>36</td>
<td>0.750</td>
</tr>
</tbody>
</table>
Table 2. Mean reproductive output of golden whistlers at Toolangi State Forest. Mean number of nests initiated, eggs, nestlings and fledglings are shown for both each nest and each pair per season. Values after means indicate standard errors.

<table>
<thead>
<tr>
<th></th>
<th>Mean per nest (range)</th>
<th>Mean per pair</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 95)</td>
<td>(n = 39)</td>
</tr>
<tr>
<td>Nesting attempts</td>
<td>1.70 ± 0.09</td>
<td></td>
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<tr>
<td>Eggs</td>
<td>2.2 ± 0.1 (2 – 3)</td>
<td>3.45 ± 0.18</td>
</tr>
<tr>
<td>Nestlings</td>
<td>1.4 ± 1.1 (0 – 3)</td>
<td>2.28 ± 0.19</td>
</tr>
<tr>
<td>Fledglings</td>
<td>0.7 ± 0.1 (0 – 3)</td>
<td>1.16 ± 0.13</td>
</tr>
</tbody>
</table>
Figure 1. Proportion of males and females banded in 2001/2002 and 2002/2003 that
returned to the study area at Toolangi State Forest in subsequent years. Values above bars
indicate number of returned individuals within each group.
Figure 2. Fate of nests initiated in each month. All data for the three breeding seasons (2001/02 – 2003/04) are combined. Values above bars indicate sample sizes.
Chapter 3

Sex-specific selection pressures on male singing strategies in golden whistlers

van Dongen (in press) Behaviour

Abstract

Male song has been shown to have two major functions: repelling rivals and attracting mates. However, the degree to which male singing strategies have been influenced by selection pressures imposed by females versus males continues to be debated. I use a combination of song recordings and playbacks to investigate how male competition and female choice may have shaped song structure and behaviour in migratory golden whistlers (Pachycephala pectoralis). Song output was low during territory establishment but increased dramatically when breeding commenced. Singing during the female fertile period may be an effective paternity guard against potential cuckolders. During this period, males decreased their song output when more neighbouring females were fertile, possibly because they were unable to concurrently guard mates and pursue extra-pair fertilisations. However, high singing rates were maintained after the fertile period, despite mate guarding no longer being necessary. Song repertoire size increased with male age and may signal male quality. Repertoires included two broad song-types: simple ‘whistle’ songs and more complex ‘melodic’ songs. Whistle songs were often sung by breeding males and may be more effective at broadcasting information at long-distances. In contrast, melodic songs were commonly sung in close-range male encounters and may contain more information about signaller quality. During vocal contests, males exhibited a range of countersinging behaviours, including song-type switching, song-type and frequency-matching, and song overlapping, which may reflect a hierarchical mode of signalling aggression. Each sex therefore appears to have imposed different selection pressures on the vocal output of male golden whistlers.
Introduction

Male bird song is an important signal used to efficiently convey information to mates or rival males (Catchpole and Slater 1995). Many components of song structure and singing behaviour are highly conserved between individuals of a species, providing a reliable cue for discrimination by conspecific and heterospecific individuals (e.g. Soha and Marler 2001; Hauber et al. 2002). However, there is also considerable within-species variation, both among individuals and across time scales. Variation in song parameters among individuals may signal the broadcasting individual’s quality (Catchpole and Slater 1995), while temporal variation in song may occur if males synchronise their singing output with the fertile period of females, or adjust output relative to perceived intruder threat during male-male vocal contests (Møller 1991b; Todt and Naguib 2000).

Male acoustic signals are generally directed at two classes of receivers: females and rival males, and these may exert different selection pressures on song characteristics. Individual variation in singing behaviour or song complexity may reflect aspects of male quality such as age, genetic fitness or past developmental stress, and these cues are therefore often used by females in mate choice decisions (Searcy 1992; Hasselquist et al. 1996; Spencer et al. 2004). Males which use song for mate attraction often maximise their singing output during periods in which females are unpaired or, in species in which extra-pair fertilisations are common, during the entire period in which females are receptive (Møller 1991b). Males with the highest song output typically gain more paternity, via increased extra-pair fertilisations with neighbouring females and decreased risk of within-pair cuckoldry (Hasselquist et al. 1996; Møller et al. 1998; Forstmeier et al. 2002).

In contrast, variation in traits used in male-male contests is typically low across the breeding season but increases relative to the threat posed by conspecifics during male-male song contests (Todt and Naguib 2000; Naguib 2005). During an interaction, the timing and patterning of singing behaviour relative to the opponent reveals the individual’s social status or willingness to escalate the contest. The plasticity of this interactive singing allows signals to be directed towards specific individuals during vocal contests. Male signalling output may vary based on a hierarchy of behaviours. High rates of switching between song-
types are often used as a low-level form of aggression in the early stages of an encounter (Kramer et al. 1985; Nielsen and Vehrencamp 1995; Horn and Falls 1996). Once aggression levels increase, song-type matching and song overlapping of rival songs become more common (Vehrencamp 2001; Otter et al. 2002; Mennill and Ratcliffe 2004). Males may also vary the structure of songs used during these contests (Mennill and Ratcliffe 2004) or use specific song-types in different contexts. For example, male nightingales (Luscinia megarhyncos) use particular song-types for long-range communication because the simple acoustic structure of these songs minimises signal degradation with distance (Naguib et al. 2002).

Here I describe variation in the song of the golden whistler (Pachycephala pectoralis), a highly vociferous and partially-migratory passerine inhabiting forests throughout southeastern Australia. By documenting individual and temporal variation in male golden whistler song complexity and singing behaviour, I speculate on the likely receivers of each signal. Signals used in mate choice are expected to increase dramatically during the breeding period, while males should augment traits used in male competition during vocal contests with neighbouring rivals. I conclude by discussing how selection pressures from male competition and mate attraction may have differentially shaped variation in male singing strategies.

Methods

Study site and species

This study was carried out between September 2001 and February 2004 at Toolangi State Forest, Victoria, Australia (37°31’ S, 145° 32’E). The predominant canopy vegetation is mountain ash (Eucalyptus regnans), with a variable understorey dependent on local topography (Chapter 2; van Dongen and Yocom 2005).

The golden whistler is a socially monogamous passerine that frequently engages in extra-pair matings (Chapter 5). Sexes are dichromatic, with males possessing brightly coloured plumage and females a uniform grey-brown plumage. Males typically acquire full adult
plumage in their third year, before which they resemble females. Males in their first year of full adult plumage can be distinguished from females by the presence of traces of grey throughout their plumage. The species is migratory at the study site, with individuals arriving in early to mid-September and departing in late April. Both sexes within a pair vigorously defend their territory throughout this time (Chapter 2; van Dongen and Yocom 2005).

Individuals were captured and individually marked with a metal band supplied by the Australasian Bird and Bat Banding Scheme, and a unique combination of three colour bands. The population was monitored daily throughout the breeding season. Twenty-five banded pairs were monitored in 2001, 27 in 2002 and 24 in 2003. After locating an individual, I noted its behaviour and position using a set of reference points spread throughout the study site (97 reference points over 106 hectares). Male territories were then mapped using the geographic information system software package ArcView GIS 3.2 (Environmental Systems Research Institute 1999). Nests were located by following an individual until the nest was visited. Active nests were monitored every two or three days to determine the breeding status of the pair.

*Song recordings*

In 2001, recordings were made using a Sony TCD-D8 Digital Audio Tape recorder and Sennheiser ME67 unidirectional microphone. Most recordings were made at a distance of between 5 and 30 metres from the focal bird. I opportunistically recorded song time budgets throughout the breeding season (mean song time budget duration: 21 min 19 secs ± 11 min 34 secs SD). In 2002, I documented individual variation in singing rates by following an individual for a 30-minute period and counting all songs sung by ear. In 2003, recordings were made in conjunction with simulated territory intrusion experiments involving live caged decoy birds and song broadcast from speakers (Chapter 7). I made recordings using a Sony TC-D5 pro-stereo cassette recorder and Sennheiser ME67 unidirectional microphone.
Song analysis

Songs were digitised using Syrinx-PC sound analysis software (J. Burt, University of Washington, Seattle, USA) at a sampling rate of 22050 Hz and using a 16-bit sound card. Songs were then displayed as frequency spectrograms and song categorisation performed via visual inspection (Bolhuis et al. 1999; Ficken et al. 2000; Latruffe et al. 2000; Ballentine et al. 2003). Element-types (defined as a continuous tracing on a spectrogram) were categorised by overlaying elements and determining levels of similarity. Each song was then coded according to a hierarchical structure from individual elements to phrases (groups of elements in a specific syntactical order) to song. Similar song-types could easily be determined by the syntactical order of elements. The number of elements was not used as a criterion to assign song-types to categories as some song-types (within the ‘whistle’ song category – see Results) exhibited high variation in the number of repetitions of introductory elements.

Song structure

A range of song structure parameters was measured for each song, including song duration (mS), number of elements, element diversity (number of different elements within a song as a proportion of the total number of elements), element switch rate (the number of switches between element-types within a song as a proportion of the total number of possible switches within that song), element repeat rate (the number of times specific element-types are repeated in succession within a song as a proportion of the total number of possible repeats) and minimum and maximum frequency (kHz).

Song repertoires

Male song repertoires were determined by calculating the total number of different song-types sung by an individual male for all recordings in a given year. To ensure that the
majority of song-types were logged for each male I constructed repertoire exhaustion curves by plotting the number of song-types encountered against the total number of songs sampled (Fig. 1). In subsequent analyses, only individuals whose exhaustion curves converged on saturation, by completely or nearly reaching asymptote, were used (Catchpole and Slater 1995; Ballentine et al. 2003; Kipper et al. 2004).

**Song-type sharing between neighbours**

The number and identity of song-types sung by each of 15 males recorded in 2001 was determined and compared to song-types sung by all other males. Each song-type was assigned a unique code. A song-type sharing coefficient between all possible male-male pairs was then calculated using the following equation: $SC = C/((A+B)-C)$, where $A$ and $B$ are the number of song-types sung by male A and B, and $C$ is the number of shared song-types between the males (Grießmann and Naguib 2002). Thus the sharing coefficient can range from 1 for complete sharing of song repertoires to 0 for no sharing. Sharing coefficients were then regressed against distance between male territories or compared between neighbours and non-neighbours.

**Song variation across breeding stages**

To investigate how song structure varied across the breeding season I analysed a sample of songs from males recorded during different breeding stages in 2001. For each whistler pair I defined four stages within the breeding season: pre-breeding (commencing when males first arrived at the site and ending when nest building was first observed for that pair), nest building (commencing when the female was first seen collecting nesting material for a nest and ending when the penultimate egg was laid), incubation (commencing after the final egg was laid in a clutch until egg hatching) and post-breeding (after all breeding activities had ceased, including the feeding of fledglings, and before individuals departed the study site). A mean of $8.9 \pm 1.9$ SD songs were analysed per male (depending on the quality of the
recordings) and in most cases each male was only used once in the analysis: in one case a male was used for both incubation and post-breeding stages.

To examine how song repertoires vary throughout the season, I calculated the repertoire sizes of a sample of males recorded during the breeding stages of 2001 (6.7 ± 2.1 SD males/breeding stage). However, due to the low song rates of males during the post-breeding stage, I was not confident that I had sampled the total repertoire size of each male during this period and so only included the first three breeding stages in the analysis.

Song rate variation was investigated in 2002 by compiling song time budgets for males across the four breeding stages throughout the season. I attempted to compile song time budgets for each male across all four breeding stages, but this was not possible for all males (mean number of breeding stages per male: 3.7 ± 0.5 SD stages, n = 18).

*Song variation during simulated territorial intrusions*

I collected data on song variation during male-male vocal contests by using playbacks to simulate territorial intrusion by rival males and provoke song duels (n = 32). Each playback track consisted of a five-minute loop broadcasting five different song-types recorded from a single male from a foreign population (see Chapter 7). To avoid pseudoreplication, recordings from different males were used for each track. A trial consisted of locating an individual and recording all vocalisations made by the focal male during a 5-minute pre-playback period. A caged live decoy male was then placed within 40 metres of the focal male (mean distance from focal individual: 20.3 ± 9.2 SD m) together with a Sony CDX-L460X portable car stereo with Realistic 30 W speakers. Songs were broadcast at a volume approximating the natural intensity of golden whistler song (50 dB at 10 m, Lutron SL-4001 sound level meter). During the 5-minute playback period, all vocalisations made by the male were recorded. I also estimated male distance from the cage using a three-dimensional zoning system. Nine different zones were identified (in a 3 x 3 design) from
zone 1 (within 5 m from the cage, both horizontally and vertically) to zone 9 (further than 20 m from the cage horizontally and more than 15 m above the cage; see Chapter 7 for details). Male zone location was dictated onto the cassette recorder. Male distance from cage was later estimated by calculating the length of the vector spanning the top of the cage to the middle of each zone (i.e. distance from cage = \(\sqrt{d^2 + h^2}\), where \(d\) = the zone’s horizontal distance from cage and \(h\) = the zone’s height above cage). A 5-minute post-playback period followed in which all male vocalisations were recorded.

I measured structural features of all songs recorded during each period. I also recorded four behavioural variables throughout the trials: song switching rate (how many times a male switched between different song-types as a proportion of total number of possible switches), song alternating (when a male sang within one second of the broadcast song), song overlapping (when a male temporally overlapped the broadcast song with his own song) and song-type matching (when a male replied to the broadcast song with the same song-type as broadcast by the speaker).

*Statistical analysis*

All statistical procedures were carried out using either SPSS 12.0 (SPSS Inc, Chicago, Illinois, USA) or Genstat 7.0 (Lawes Agricultural Trust, 2003). Data were tested for normality and log-transformed where necessary. *Post hoc* analyses for all significant ANOVAs were performed using Tukey tests. In analyses involving multiple tests I conducted Bonferroni corrections. To reduce the subsequent increased risk of type II errors, I increased the alpha level to 0.1 for these tests only (Wright 1992; Chandler 1995). Data are presented as means and standard deviations unless otherwise stated.

To investigate how males vary song structure during specific countersinging behaviours, I compared structural features of all songs sung while performing each type of behaviour with that of a random sample of songs, taken as an estimate of the ‘population mean’. This random sample was obtained from songs recorded from all males during the simulated intrusions when not engaged in any of the countersinging behaviours. I ensured that sample
sizes of the singing behaviour category and random sample category were similar to avoid the need for non-parametric tests. Distance from threat during these behaviours was analysed in a similar fashion.

Results

**General song characteristics**

**Song structure**

The typical song of male golden whistlers consists of a series of introductory elements sung within a narrow frequency bandwidth and terminating in a ‘whip-crack’ element that rapidly sweeps across a wide frequency range. Two broad classes of song could be identified: ‘whistle’ songs, which commenced with a series of pure-tone whistle elements, and ‘melodic’ songs with much higher element variability (Fig. 2). Overall, 47.7% ($n = 843$) of songs were whistle songs. Across all song-types, songs were on average $1\,097 \pm 528$ mS long, containing $5.7 \pm 2.6$ elements and $3.6 \pm 1.3$ element-types. Structural features of whistle songs differed significantly from melodic songs (Fig. 3). Whistle songs were more repetitive and less variable than melodic songs: they had higher element-type repetition (whistle: $0.49 \pm 0.17$ repeats/element; melodic: $0.01 \pm 0.03$ repeats/element; $F_{1,186} = 854.285$, $p < 0.001$) and lower element diversity (whistle: $0.53 \pm 0.18$ element-types/element; melodic: $0.82 \pm 0.20$ element-types/element; $F_{1,186} = 102.011$, $p < 0.001$). Whistle songs were longer than melodic songs (whistle: $1\,332 \pm 520$ mS; melodic: $956 \pm 483$ mS; $F_{1,186} = 24.245$, $p < 0.001$). However, they did not contain more elements (whistle: $6.1 \pm 2.6$ elements; melodic: $5.6 \pm 2.6$ elements; $F_{1,186} = 1.756$, $p = 0.187$). Instead, fewer elements were sung per unit time (whistle: $4.9 \pm 1.7$ elements/second; melodic: $6.0 \pm 1.0$ elements/second; $F_{1,186} = 30.986$, $p < 0.001$). Whistle songs also had a higher maximum frequency (whistle: $7.1 \pm 0.8$ kHz; melodic: $6.8 \pm 0.9$ kHz; $F_{1,186} = 6.015$, $p = 0.015$) and lower minimum frequency (whistle: $1.5 \pm 1.2$ kHz; melodic: $1.7 \pm 0.3$ kHz; $F_{1,186} = 31.087$, $p < 0.001$) and thus covered a larger frequency bandwidth than melodic songs (whistle: $5.6 \pm 0.8$ kHz; melodic: $5.1 \pm 0.1$ kHz; $F_{1,186} = 20.478$, $p < 0.001$). These correlations all remained significant after Bonferroni correction.
Song repertoires

Male song repertoires were revealed slowly, as males sang with eventual variety, singing a single song-type for a number of repetitions before switching to another song-type. Male repertoire sizes ranged from three to 22 song-types (mean: 10.1 ± 4.7 song-types, n = 17). Adult males did not have larger song repertoires than males in their first year of adult plumage (adult: 10.3 ± 3.8 song-types, n = 13; first year adults: 7.6 ± 3.4 song-types, n = 5; F1,16 = 1.980, p = 0.179). A comparison of repertoires of males who were recorded in both 2001 and 2003 suggested repertoire size increases with age (mean repertoire size: 2001: 8.8 ± 4.4 song-types; 2003: 14.2 ± 7.1 song-types; paired t-test: t = -2.527, d.f. = 4, p = 0.065). The mean number of songs added to a male’s repertoire per year was 2.7 ± 1.3 songs/year (range: 0.5 – 7.5 songs/year). This pattern is unlikely to be due to differences in sampling effort between years, because the analysis only included males for which I was confident that I had recorded the majority of song-types.

Song-sharing between neighbours

Males shared an average of 12.2 ± 8.0 % of their song repertoires with each male within the study site. A small amount of the variation in song-type sharing between individuals was explained by distance between individuals - males in close proximity shared more song-types than males defending distant territories (GLMM: distance effect: -0.009 ± 0.003, d.f. = 1, Wald = 14.32, p < 0.001). There was also a trend towards neighbouring males sharing more song-types than non-neighbours (mean song sharing between immediate neighbours: 15.1 ± 10.0 %, non-neighbours: 11.5 ± 7.3 %; F1,102 = 3.530, p = 0.063). Thus male repertoires possessed, on average, 1.5 song-types that were also sung by an immediate neighbour (15.1% of the mean repertoire size of 10.1 songs-types/male).
**Song variation across breeding stages**

**Song structure**

Male song structure remained constant throughout the pre-breeding and breeding stages. However, when breeding had ceased, males typically sang shorter songs with higher element diversity ($F_{3,25} = 7.080, p = 0.001; F_{3,25} = 6.962, p = 0.001$ respectively; Table 1). The minimum frequency of these songs increased compared to the rest of the season ($F_{3,25} = 5.426, p = 0.005$), but maximum frequency did not vary ($F_{3,25} = 2.272, p = 0.105$). Males did not vary the element-type switching rate throughout the season ($F_{3,25} = 0.323, p = 0.809$) but nor the element-type repetition rate ($F_{3,25} = 3.272, p = 0.038$, non-significant after Bonferroni correction).

**Song repertoire**

The number of different song-types sung by males during singing bouts did not vary across the season (pre-breeding: $7.0 \pm 2.6$ song-types; nest building: $8.6 \pm 3.6$ song-types; incubation: $7.9 \pm 2.7$ song-types; $F_{2,18} = 0.427, p = 0.659$). However, the proportion of whistle songs sung by males did. Males sang whistle songs $43.1\%$, $40.8\%$ and $43.8\%$ of the time during the pre-breeding, nest building and incubation stages respectively. This dropped to $11.1\%$ after the completion of breeding ($\chi^2 = 215.42$, d.f. = 3, $p < 0.001$).

**Song rate**

Song rates varied considerably across the breeding stages ($F_{3,59} = 49.039, p < 0.001$; Fig. 4). Song rates of males that had recently arrived at the study site were relatively low. However, song rates increased dramatically once the birds started breeding. Males tended to sing at slightly higher rates while incubating in comparison to the nest building stage ($F_{1,28} = 3.287, p = 0.081$). Song rates dropped to almost zero after the cessation of breeding.

Throughout nest building, males tended to sing at lower rates in the presence of a higher number of fertile females (defined as females building nests; overall model: $F_{2,17} = 9.009$, p
number of receptive females: $F_{1,17} = 3.448, p = 0.081$; total number of neighbouring females: $F_{1,17} = 7.684, p = 0.013$; Fig. 5). This relationship disappeared during the egg incubation stage (overall model: $F_{2,13} = 0.714, p = 0.508$; number of receptive females: $F_{1,13} = 0.019, p = 0.892$; total number of neighbouring females: $F_{1,13} = 1.329, p = 0.270$).

**Song variation during simulated territorial intrusions**

**Song structure**

Males did not change any aspects of their song structure during or after an intrusion by another male. However, during the intrusion a small percentage of variation in song structure was explained by the proximity of the territory holder to the intruder. The territory-holding males sang with a higher element diversity ($r^2 = 0.078, F_{1,78} = 6.641, p = 0.012$) and element switch rate the closer they were to the intruder ($r^2 = 0.065, F_{1,78} = 5.505, p = 0.021$). Song length did not vary with distance ($r^2 = 0.010, F_{1,77} = 0.762, p = 0.385$). No variation in structural features of whistle songs was explained by distance from the threat, including frequency of whistle elements ($F_{1,34} = 0.216, p = 0.645$), whistle element length ($F_{1,34} = 1.382, p = 0.248$) or inter-element pause lengths ($F_{1,34} = 2.836, p = 0.102$).

**Song repertoire**

Controlling for the number of songs sung during each period of the simulated territory intrusions, males did not increase the number of song-types sung during or after an intrusion (song-types sung: pre-playback = $0.22 \pm 0.04$ song-types/song sung; playback = $0.23 \pm 0.03$ song-types/song sung; post-playback = $0.23 \pm 0.06$ song-types/song; $F_{2,47} = 0.005, p = 0.995$). During an intrusion, males sang more melodic songs in comparison to before and after the intrusion (before: $0.47$ melodic songs/song sung, during: $0.58$ melodic songs/song sung, after: $0.46$ melodic songs/song sung, $\chi^2 = 17.56$, d.f. = 2, $p < 0.001$). When focal individuals were classified as either ‘near’ the simulated intruder (in four
closest playback zones; mean distance = 10.7 ± 5.3 m) or ‘far’ (in four furthest playback zones; mean distance = 30.3 ± 5.2 m), males closer to the simulated intrusion were more likely to sing melodic songs (proportion of songs that were melodic songs: near: 0.69 ± 0.08 melodic songs/song sung; far: 0.40 ± 0.20 melodic songs/song sung, F$_{1,6}$ = 7.039, p = 0.035).

Song rate

Song rate did not vary across the three playback periods (before: 5.2 ± 2.9 songs/min, during: 5.4 ± 3.2 songs/min, after: 5.6 ± 3.7 songs/min; F$_{2,91}$ = 1.830, p = 0.166).

Song switching

Singing males cycled through more song-types during and after the intrusion compared to before (0.43 ± 0.20 [during], 0.37±0.23 [after] and 0.25±0.20 [before] switches per songs; F$_{2,91}$ = 5.647, p = 0.005).

Song alternating

During the simulated intrusions, males often sang in an alternating fashion with the speaker (Fig. 6). Males typically sang shorter songs when song-alternating with the songs broadcast from the speaker (F$_{1,179}$ = 6.534, p = 0.011). These songs also had a higher element diversity (F$_{1,179}$ = 20.078, p < 0.001), greater element-type switch rate (F$_{1,179}$ = 31.578, p < 0.001) and a lower element-type repeat rate (F$_{1,179}$ = 43.126, p < 0.001) than the population mean (Table 2). Male song alternating rates did not vary with distance from intruder (F$_{1,179}$ = 1.144, p = 0.286).

Song overlapping

Song overlapping occurred when the focal male sang over the top of the song broadcast by the speaker (Fig. 6). Males overlapped 3.8% (91/2396) of the songs broadcast from the speaker with their own song. The songs males used to overlap the playback songs with
were shorter ($F_{1,171} = 5.120, p = 0.025$), had higher element diversity ($F_{1,171} = 11.226, p = 0.001$), a greater element-type switch rate ($F_{1,171} = 14.357, p = 0.008$) and lower element-type repeat rate ($F_{1,171} = 25.794, p < 0.001$) than the population mean (Table 2). When males overlapped the speaker’s song, they were 5.8 m closer to the simulated intruder than average (distance from intruder: overlapping male = 17.6 ± 10.2 m, population mean = 23.4 ± 10.1 m; $F_{1,71}=14.270, p < 0.001$).

**Song-type matching**

Of all songs sung by males, 0.9% (22/2396) were matched replies to the playback songs (Fig. 6). However, this is likely to be an underestimate of natural song-type matching rates, since the playback songs used were from a foreign population and so may not have been in the focal males’ repertoire. When a male matched the playback song with the same song, his song was shorter ($F_{1,41} = 7.678, p = 0.008$), had higher element diversity ($F_{1,41} = 14.765, p < 0.001$) and greater element switch rate ($F_{1,41} = 5.330, p = 0.026$) and lower element repeat rate ($F_{1,41} = 8.582, p = 0.006$; Table 2) than the population mean. Song-type matching rates did not vary with distance from the stimulus ($F_{1,41} = 2.809, p = 0.101$).

Males often sang whistle songs in reply to whistle songs broadcast by the speaker. The frequency of the whistle elements broadcast by the speaker had a significant effect on the frequency of whistles in the focal males’ replies ($r^2 = 0.538, F_{1,13} = 15.155, p = 0.002$). When a male replied to a whistle song broadcast by the speaker, the frequency of the whistle elements of his song were typically at or below that broadcast whistle frequency (Fig. 7).

**Discussion**

Male golden whistlers sang two structurally different song-types which were sung in different contexts. Whistle songs appeared to be broadcast to conspecifics over longer distances, while the structurally more complex melodic songs were more often used during close-range male encounters. Singing rates fluctuated considerably throughout the breeding season and peaked during the nest building and incubation stages. During nest building, but
not incubation, male singing rates decreased in the presence of a higher number of fertile neighbouring females. Singing rates did not increase during or after an intrusion from a neighbouring male. Instead, a hierarchical suite of countersinging behaviours may convey information to conspecific males about an individual’s willingness to escalate a contest.

**Singing behaviour and reproductive success**

Male golden whistlers arriving at the breeding site initially had low song output. Singing may be unimportant for mate attraction at this stage as males usually re-pair with the same partner each year (Chapter 2; van Dongen and Yocom 2005). However, song output increased dramatically during the female fertile period (i.e. during nest building). This pattern is common in passerines, and has been explained as a means to guard against potential brood cuckolders. If females accept fertilisations from outside the pair bond, then males should increase their reproductive fitness by guarding their female during the period when she is receptive to fertilisations (Møller 1991b). During this period, males often adjust song features associated with viability to announce their quality (Slagsvold *et al.* 1994; Welling *et al.* 1995; Ballentine *et al.* 2003). For example, the costs of singing at high rates, such as an inability to simultaneously forage and sing, can presumably only be borne by high quality individuals (Gil and Gahr 2002). Territories in which males broadcast a high song output should be avoided by potential cuckolders as these males may possess superior competitive skills and be paired to females that are less likely to accept extra-pair copulations. Cuckolders should instead focus on territories with little song announcement for extra-pair forays.

Interestingly, during their partner’s fertile period, males sang at lower rates in the presence of higher numbers of fertile females. The mean population singing rates also tended to be lower during nest building, when more females were simultaneously fertile, than during incubation. This may reflect a conflict between the inability to concurrently pursue extra-pair fertilisations and sing at high rates to guard against within-pair cuckoldry (Chuang-Dobbs *et al.* 2001), although males may still adjust their mate-guarding behaviour relative to their ability to attract extra-pair fertilisations and probability of being cuckolded (Kokko and Morrell 2005). This is supported by the fact that males singing at high rates during the
female fertile period (possibly as a mate-guarding tactic in response to a greater probability of being cuckolded) sired fewer young (Chapter 5). During incubation, the need to guard against cuckolders is presumably no longer necessary and the relationship between singing rates and number of fertile neighbouring females disappears. However, despite mate guarding no longer being necessary subsequent to egg laying, high singing rates are still maintained and actually increase slightly. This pattern suggests that singing subsequent to the female fertile period may advertise male quality to potential extra-pair mates. Singing rates during this period were unrelated to male reproductive success (Chapter 5) but may instead contribute to a multi-stage mate selection process, whereby females initially emphasise a readily assessable long-range signal to select a subset of potential mates and then switch to a more reliable, short-range signal (e.g. plumage colour). Similar multi-stage selection processes have been reported in other species (e.g. Jackson’s widowbird, *Euplectes jacksoni*; Andersson 1989) and may, in part, explain the persistence of multicomponent sexual displays in this species. Nevertheless, if this were the case, then singing rates would be expected to be at least weakly related to male extra-pair reproductive success. A more likely alternative is suggested by male singing rates being positively related to territory size (Chapter 5). A high song output may be a response to the increased need for resource defence during the energetically demanding period of nestling provisioning (Bryant 1988; Bryant and Tatner 1991).

Due to the multifaceted nature of song, singing rates are not the only signal that may enhance male reproductive success. For example, high intrapopulation variation in song repertoire sizes exists and may in part signal male age, as males augment their song repertoires each year. Repertoire size seems to be unimportant for male competition, as males did not increase the number of song-types sung in response to male intruders. Instead, song repertoire may be used as a cue during female mate choice decisions. Indeed, male golden whistlers possessing large song repertoires are less likely to be cuckolded and sire more offspring, even after controlling for male age (Chapter 5). In many species, females preferentially mate with males with more complex song (e.g. Searcy 1992; Hasselquist *et al.* 1996), which may reflect genetic quality (Hasselquist *et al.* 1996) or past developmental stresses (Nowicki *et al.* 1998; Spencer *et al.* 2003).
Singing behaviour and close-range male interactions

During an intrusion, resident males typically increased the number of switches between different song-types and often matched the song-types or overlapped the songs broadcast by the speaker. These response patterns are common amongst passerines and typically reflect male dominance and motivation to persist with a territorial dispute (Todt and Naguib 2000; Naguib 2005). Different countersinging behaviours often reflect increasing levels of signaller aggression, allowing graded signalling during male-male interactions. Golden whistler males increased switching rates during intrusions by rivals. The probability of switching between songs was, however, unrelated to distance from the threat. In many species, males increase rates of song-type switching during early stages of male-male contests (e.g. Kramer et al. 1985; Nielsen and Vehrencamp 1995). Since song-type switching does not require interactive singing with the intruder, this behaviour may represent a more general, non-directional signal of aggression.

During escalated male-male encounters, individuals often display higher levels of aggressive behaviour such as song-type matching or overlapping (Kramer et al. 1985; Nielsen and Vehrencamp 1995; Horn and Falls 1996). Male whistlers overlapped more songs sung by the intruder when they were closer to that individual. This pattern may be a reflection of both higher levels of aggression as the resident male approaches the intruder, and the increased efficiency of overlapping intruder’s songs at close range. Song overlapping is considered aggressive because the transmission of the overlapped signal is masked and information within the original signal is rendered undecipherable. The cost of having one’s signal masked increases for close-range encounters because at a distance, the overlapping song will be attenuated to a relatively low amplitude, rendering masking effects less efficient (Todt and Naguib 2000).

Song-type matching is an additional means by which individuals can direct their signals to particular receivers (e.g. Burt et al. 2001; Otter et al. 2002). However, song-type matching requires a significant degree of repertoire sharing between neighbours. Many species have a large and variable song repertoire, resulting in low repertoire sharing between neighbours and rendering song-type matching difficult. Frequency matching of particular parts of song
is a unique means by which such species can match intruder songs without increasing song repertoires (Naguib et al. 2002; Naguib 2005). Golden whistlers only share a small portion of their repertoires with neighbours (an average of 1.5 shared songs between immediate neighbours). Due to the simple structure of whistle songs, males can match the frequency of the whistle elements sung by intruders, permitting more song matching opportunities. Therefore, in this species, frequency matching may complement song switching, matching and overlapping, allowing complex, multilevel interactions between individuals, regardless of degree of repertoire sharing between neighbours.

*Whistle songs and long-range communication*

Given the importance of song in long-distance communication, selection may favour modifications of song structure that maximise efficient signal transmission between signallers and receivers (Morton 1975; Wiley and Richards 1982; Wiley 1991). The scattering surfaces of vegetation are a major cause of inefficient song transmission, typically resulting in a loss of valuable information (Morton 1975). However, the temporal and frequency-related aspects of song structure vary predictably in how they are affected by propagation through a dense habitat. For example, whistle songs contain a high number of pure tone elements with long inter-element pauses, and are therefore predicted to suffer less from degradation with distance (Wiley 1991; Slabbekoorn et al. 2002; Slabbekoorn and Smith 2002). Hence, the simple acoustic structure of whistle songs may render them more efficient in long-distance communication. Similar whistle songs are sung by male nightingales and are used during long-range communication with both sexes (Naguib et al. 2002). Male golden whistlers sang more whistle songs in long-range interactions, but decreased the use of whistle songs after the cessation of breeding, suggesting a potential role in announcement to potential extra-pair mates. However, the number and structure of whistle songs sung by a male was unrelated to reproductive success (unpublished data). Thus whistle songs may be more important in long-range intraspecific communication. During general territory announcement, conspecifics are typically much further away than during elevated male-male contests: since the average territory size of golden whistlers is 2.5 hectares (Chapter 2; van Dongen and Yocom 2005), the mean distance between a singing male and a neighbour (assuming they are both in the centre of their territories) is
158 m. Thus singing more whistle songs in these long-distance interactions would maximise the efficiency of information reaching the receiver. Surprisingly, males did not vary any structural features of whistle songs in relation to their distance from the intruder that would further minimise song degradation.

As they approached the intruder, males sang more melodic songs. The complex structure of these songs renders them more suitable for close-range encounters. At these distances, information loss via song degradation will be reduced and conspecifics may pay more attention to variation in these more complex songs that cannot be distinguished at greater distances. This greater complexity may provide more information concerning the quality of the signaller in terms of social rank and level of threat (Eens 1997; Spencer et al. 2004).

Conclusions

The song of male golden whistlers is a complex, multi-faceted signal, broadcasting information to both male and female conspecifics. Male singing rates may function as an effective paternity guard against male cuckolders during the female fertile period and signal male presence to potential extra-pair mates. Likewise, song repertoire size may be an important cue used by females to assess male age and long-term quality in mate choice decisions. Females may use different song cues in social versus extra-pair mate choice decisions if the various cues are associated with different direct or indirect benefits for the females (e.g. good genes for the offspring versus larger territories). Females may also select males via a hierarchical process, selecting a subset of potential mates using song cues while still in their territory and then visiting a subset of preferred males to assess other cues in close proximity. In contrast, a hierarchy of male behaviours seems to be important during territorial disputes and appear to advertise male motivation during male-male vocal contests. Such interactive vocal contests allow males to direct their signal to specific individuals and grade their response in relation to the relative threat of the rival. Thus the song of male golden whistlers appears to be shaped differentially via male and female selection.
**Table 1.** Variation in song structure features sung by male golden whistlers across the four breeding stages at Toolangi State Forest.

The song structural features are song minimum and maximum frequency (kHz), total number of elements, element diversity (element-types/element; number of different elements within a song as a proportion of the total number of elements), element switch rate (switches/element; the number of switches between element-types within a song as a proportion of the total number of possible switches within that song) and element repeat rate (repeats/element; the number of times specific element-types are repeated in succession within a song as a proportion of the total number of possible repeats). Means are presented with standard errors. * Non-significant after Bonferroni adjustment.

<table>
<thead>
<tr>
<th></th>
<th>Pre-breeding</th>
<th>Nest building</th>
<th>Incubation</th>
<th>Post-breeding</th>
<th>F&lt;sub&gt;numerator df, denominator df&lt;/sub&gt;</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Frequency</td>
<td>7.38 ± 0.11</td>
<td>7.16 ± 0.10</td>
<td>6.91 ± 0.16</td>
<td>7.27 ± 0.42</td>
<td>2.272&lt;sub&gt;3,25&lt;/sub&gt;</td>
<td>0.105</td>
</tr>
<tr>
<td>Minimum Frequency</td>
<td>1.57 ± 0.04</td>
<td>1.49 ± 0.02</td>
<td>1.59 ± 0.06</td>
<td>1.73 ± 0.03</td>
<td>5.426&lt;sub&gt;3,25&lt;/sub&gt;</td>
<td>0.005</td>
</tr>
<tr>
<td>Number of elements</td>
<td>6.5 ± 0.9</td>
<td>7.0 ± 0.5</td>
<td>8.2 ± 0.9</td>
<td>3.8 ± 0.2</td>
<td>7.080&lt;sub&gt;3,25&lt;/sub&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>Element diversity</td>
<td>0.64 ± 0.08</td>
<td>0.64 ± 0.02</td>
<td>0.57 ± 0.06</td>
<td>0.90 ± 0.03</td>
<td>6.962&lt;sub&gt;3,25&lt;/sub&gt;</td>
<td>0.001</td>
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<tr>
<td>Element switch rate</td>
<td>0.56 ± 0.05</td>
<td>0.63 ± 0.05</td>
<td>0.61 ± 0.06</td>
<td>0.61 ± 0.04</td>
<td>0.323&lt;sub&gt;3,25&lt;/sub&gt;</td>
<td>0.809</td>
</tr>
<tr>
<td>Element repeat rate</td>
<td>0.26 ± 0.07</td>
<td>0.21 ± 0.04</td>
<td>0.24 ± 0.07</td>
<td>0.07 ± 0.03</td>
<td>3.272&lt;sub&gt;3,25&lt;/sub&gt;</td>
<td>0.038*</td>
</tr>
</tbody>
</table>
Table 2. Structural variation in male golden whistler song while performing different countersinging behaviours. The song structural features are total number of elements, element diversity (element-types/element; number of different elements within a song as a proportion of the total number of elements), element switch rate (switches/element; the number of switches between element-types within a song as a proportion of the total number of possible switches within that song) and element repeat rate (repeats/element; the number of times specific element-types are repeated in succession within a song as a proportion of the total number of possible repeats). Asterisks signify significant differences in song structure between an estimate of the population mean and songs sung during specific countersinging behaviours at the 0.05 (*) and 0.001 (**) levels. All correlations remained significant after Bonferroni corrections. Means are presented with standard errors.

<table>
<thead>
<tr>
<th></th>
<th>Population mean</th>
<th>Song alternating</th>
<th>Song matching</th>
<th>Song overlapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of elements</td>
<td>6.0 ± 0.3</td>
<td>5.1 ± 0.2*</td>
<td>4.4 ± 0.4*</td>
<td>5.1 ± 0.3*</td>
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<tr>
<td>Element diversity</td>
<td>0.64 ± 0.03</td>
<td>0.79 ± 0.02**</td>
<td>0.86 ± 0.04**</td>
<td>0.76 ± 0.03**</td>
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<tr>
<td>Element switch rate</td>
<td>0.52 ± 0.03</td>
<td>0.71 ± 0.17**</td>
<td>0.64 ± 0.17*</td>
<td>0.65 ± 0.02**</td>
</tr>
<tr>
<td>Element repeat rate</td>
<td>0.27 ± 0.03</td>
<td>0.06 ± 0.01**</td>
<td>0.11 ± 0.04*</td>
<td>0.09 ± 0.02**</td>
</tr>
</tbody>
</table>
Figure 1. Song repertoire exhaustion curves for a sample of male golden whistlers recorded at Toolangi State Forest in 2001 and 2003. To increase clarity, not all curves are shown.
Figure 2a. Examples of ‘whistle’ songs sung by male golden whistlers at Toolangi State Forest. Whistle songs typically commence with a variable number of unmodulated elements, followed by one or two terminal ‘whipcrack’ elements that rapidly sweep through a large frequency range.
Figure 2b. Examples of ‘melodic’ songs sung by male golden whistlers at Toolangi State Forest. Melodic songs are characterised by highly variable and modulated elements. Specific element-types are rarely repeated in quick succession as in whistle songs.
Figure 3. Structural differences between ‘whistle’ and ‘melodic’ songs sung by male golden whistlers. Error bars indicate standard error. Asterisks denote significant differences at the 0.05 (*) and 0.001 (**) levels. All correlations remained significant after Bonferroni correction.
Figure 4. Variation in golden whistler singing rates across the four breeding stages at Toolangi State Forest ($F_{3,59} = 49.039$, $p < 0.001$). Error bars indicate standard error.
Figure 5. The relationship between the male golden whistler singing rates during the nest building stage and the number of fertile neighbouring females (defined as those females in the nest building stage; overall model: $F_{2,17} = 9.009, p = 0.002$; number of receptive females: $F_{1,17} = 3.448, p = 0.081$; total number of neighbouring females: $F_{1,17} = 7.684, p = 0.013$). Note values represent fitted values as predicted by the General Linear Model.
Figure 6. Modes of countersinging used by male golden whistlers during simulated territory intrusions. a) song alternating, b) song overlapping, c) song-type matching. Note that in each case, the first song is that broadcast from the speaker, the second being that sung in response by the focal male.
Figure 7. The relationship between the frequency of the whistle portion of the focal male whistle song and that of the whistle song broadcast by the speaker. The solid line is the line of best fit for the data, while the dotted line indicates the line of exact frequency matches.
Isolation and characterisation of microsatellite markers for paternity assessment in the golden whistler (*Pachycephala pectoralis*: Aves)


Abstract

I isolated and characterised six novel microsatellite markers for paternity analysis in the golden whistler *Pachycephala pectoralis*, by screening an enriched genomic library using non-radioactive PCR techniques. The six loci exhibited little or no evidence of null alleles and showed high levels of polymorphism (mean $H_e=0.85$, mean number of alleles=15.2), making them suitable for paternity assessment in this species (exclusion probability of six unlinked loci = 0.9997).

Introduction

Male birds often simultaneously display several sexually selected traits, such as complex singing behaviour and elaborate plumage (Andersson 1994). Traditionally, the function of each trait has been investigated in isolation, but more recent work examines the costs and benefits of combined expression of acoustic and visual signals. This field of research is attracting increasing theoretical (Pomiankowski and Iwasa 1993; Johnstone 1995) and empirical attention (Doucet and Montgomerie 2003; Lendvai et al. 2004). Field studies suggest that multiple ornaments typically reflect different aspects of male quality or are vestiges of past selection pressures (Candolin 2003). In order to comprehend the selective importance of multiple sexually selected traits, some understanding is needed of the relationship between trait expression and a male’s reproductive success. Extra-pair fertilisations are common in birds (Westneat and Stewart 2003), thus reliable assessment of paternity is essential. Microsatellite markers are a powerful tool for this purpose.

Here I describe the isolation and characterisation of novel microsatellite markers that permit unambiguous assessment of paternity in the golden whistler (*Pachycephala pectoralis*).
pectoralis), a socially monogamous passerine endemic to Australian forests. Males possess colourful plumage and display complex singing behaviour, making this an ideal species in which to study the maintenance of multiple ornaments.

Methods

I extracted DNA from blood samples taken from golden whistlers captured at Toolangi State Forest (37° 31’ S 145° 32’ E), Victoria in Australia, using a salting-out procedure (Bruford et al. 1992). Genomic DNA was enriched for CTTT and GA repeat-containing fragments in polymerase chain reactions (PCR) after Gardner et al. (1999). Additional modifications were made as detailed in Adcock and Mulder (2002). Positive clones were amplified in 50 µl reactions and the DNA extracted using Qiaquick PCR purification kit (QIAGEN) following the manufacturer’s instructions. PCR products were sequenced commercially (SUPAMAC, Sydney, Australia).

From 173 colonies screened in the CTTT-enriched genomic library, six positive clones were sequenced. Primers were designed (Life Technologies) for five of these that contained five repeats or more and had a suitable flanking sequence. From 99 colonies screened in the GA-enriched genomic library, five positive clones were sequenced. Primers were developed for all five loci. Primer pairs that gave consistent, specific products were tested for polymorphism. One primer in each pair was manufactured with a 5’-M13 (5’CACGACGTTGTAAAACGAC) tail for use in the universal dye-labelling method described by Boutin-Ganache et al. (2001). Primer sequences and optimum annealing temperatures are listed in Table 1. I assessed polymorphism by typing at least 20 putatively unrelated individuals at each microsatellite locus. Reactions were run in 0.2 ml microtitre plate (Greiner) wells layered with a drop of mineral oil (Sigma) on a Corbett Research PC-960C thermocycler. Reactions (10 µl) contained an M13 primer (200 nM) 5’-labelled with a Beckman Coulter dye (D2, D3 or D4), and the locus-specific tailed (15nM) and untailed (200 nM) primer, 40 ng of genomic DNA and the optimal concentration of MgCl2. All PCRs used Taq polymerase (0.25 units/10 µl), MgCl2 (see Table 1 for concentrations), and a reaction buffer (10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100) and dNTPs (200 µM) supplied by Promega. A total of 40 cycles of amplification were run: one cycle of 90 s at 94
°C followed by 40 cycles of 94 °C for 20 s, the optimal annealing temperature for 20 s and 73 °C for 90 s. PCR products (0.25 μl) were electrophoresed on a Beckman Coulter 8000XL automated sequencer using the CEQ 2000XL fragment analysis kit (Beckman Coulter) according to the manufacturer’s instructions. Fragment sizes were estimated using the Beckman Coulter 8000XL fragment analysis software.

Results and discussion

Six loci were found to be polymorphic (Na = 5 – 22, mean He = 0.85; Table 1). These loci exhibited a combined exclusion probability of 0.9997. Using tests implemented in Cervus (Marshall 2001), no loci were found to deviate significantly from Hardy-Weinberg equilibrium, or show evidence of linkage disequilibrium (all p > 0.05). Ppm8 showed evidence of slight heterozygote deficiency, probably due to the presence of null alleles. When this locus was removed from the analysis, the exclusion probability remained high (exclusion probability = 0.9980). This set of loci will therefore be a valuable tool for documenting patterns of paternity in relation to the maintenance of multiple ornamentation in this species.
Table 1 Characterisation of microsatellite loci in the golden whistler. The number of individuals tested (N), number of alleles (N_a), allele size range in base pairs (bp), observed (H_o) and expected (H_e) heterozygosity and the probability of excluding a father (Ex) are listed for each locus. The final annealing temperature (T_m) is in °C and the MgCl₂ concentration in mM. The M13 prefix in the primer sequence denotes a 5’ M13 tail (CACGACGTGTGAAAAACGAC), attached to the primer sequence. Cloned sequences have been deposited with GenBank under Accession numbers AY672128-AY672133.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat motif in clone</th>
<th>Primer sequence (5’-3’)</th>
<th>N</th>
<th>T_m</th>
<th>[MgCl₂]</th>
<th>N_a</th>
<th>bp</th>
<th>H_o</th>
<th>H_e</th>
<th>Ex</th>
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<tr>
<td>Ppm1</td>
<td>(AG)₁₅GG(AG)₂</td>
<td>F: M13-GAGCTTTGTCCACTCAAGGTTTTG&lt;br&gt;R: GGAAGGAAGGAAGGACACC</td>
<td>28</td>
<td>57</td>
<td>1.5</td>
<td>5</td>
<td>120-132</td>
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<td>(AAAG)₂ATAG(AAAG)₁ACAG(AA AG)₁ACAG(AAAG)₅</td>
<td>F: M13-CAGGGTACAACCATAAGGAATTG&lt;br&gt;R: ACTTCCATGGACCAGGTTTG</td>
<td>24</td>
<td>57</td>
<td>1.5</td>
<td>12</td>
<td>155-395</td>
<td>0.67</td>
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<td>57</td>
<td>1.5</td>
<td>10</td>
<td>120-150</td>
<td>0.82</td>
<td>0.81</td>
<td>0.61</td>
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<td>Ppm8</td>
<td>(CTTT)₂T(CTTT)₅CTTA(CTTT)₅CT (CTTT)₇TT(CTTT)₂CTTC(CTTT)₁ (CTT)₆(CTTT)₄(CTT)ᵣ(CTTT)₂</td>
<td>F: M13- GAGAGCATAGGGGACTTCG&lt;br&gt;R: ACACACAGAGTGTGAGAAC</td>
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<td>55</td>
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<td>0.88</td>
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<tr>
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<td>1.5</td>
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<td>28</td>
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<td>257-329</td>
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Chapter 5

Long- and short-range signalling via multiple ornaments in the golden whistler

Unpublished manuscript

Abstract

The visual and acoustic ornaments of many male birds reliably communicate aspects of the signaller’s quality to conspecifics. In order to be reliable, ornamental signals must impose costs that cannot be borne by poor quality individuals. Given these costs, the question of why males concurrently display several such potentially costly ornamental traits is attracting increasing attention. I investigated the function of multicomponent displays in golden whistlers (*Pachycephala pectoralis*). Males possess a number of elaborate acoustic and plumage ornaments. Large throat patches and high singing rates during general territory announcement were both associated with larger territories, suggesting these signals may be used in short- and long-range intrasexual communication, respectively. Male singing rates also seemed to be important in determining reproductive success: individuals singing at high rates during the female fertile period sired fewer offspring, possibly reflecting an inability to simultaneously mate-guard and pursue extra-pair fertilisations. The carotenoid-saturated breast plumage and song repertoires of males appeared to be under strong female selection. Males with large song repertoires were less likely to be cuckolded, as were individuals possessing high-quality breast plumage. Cuckolding males also possessed larger song repertoires and ‘yellower’ breast plumage than the males they cuckolded. Females are presumably able to assess song repertoires of potential extra-pair mates at long distances, but breast colour only within close-range. Multiple sexual ornaments in this species thus efficiently convey important information to both male and female conspecifics at different distances.
Chapter 5  Multiple ornamentation

Introduction

Many male birds display conspicuous sexual signals such as colourful plumage or elaborate song. These visual and acoustic ornaments appear to reliably communicate aspects of the signaller’s quality to conspecifics, typically because they are costly to produce or maintain (Andersson 1994). Given these costs, it seems puzzling that males often concurrently display several ornamental traits. Indeed, theoretical studies predict that males should reduce costs in ornament production and maintenance by evolving a single, highly informative signal advertising male quality (Pomiankowski and Iwasa 1993; Schluter and Price 1993; Iwasa and Pomiankowski 1994, but see Van Doorn and Weissing, 2004).

Møller and Pomiankowski (1993) outlined three mechanisms that may favour the evolution of multiple display ornaments. First, various non-intercorrelated ornaments may advertise different aspects of male quality or be directed towards different receivers (multiple message signalling). For example, in red-collared widowbirds (Euplectes ardens), the red plumage collar functions in male-male competition, whereas exaggerated long tails are under strong female selection (Pryke et al. 2001; Pryke et al. 2002). Ornaments may also convey information to receivers at different distances (e.g. Andersson 1989), while in other species females are not uniform in their preferences for specific ornaments and emphasise different cues at different ages (e.g. Coleman et al. 2004). Conversely, display traits may be inter-correlated and provide information on the same aspect of male quality (back-up message signalling). If a degree of error is associated with the information content of ornaments, then estimating individual quality using a range of ornaments will minimise mistakes. Finally, ornaments may currently not signal any aspect of quality but be a vestige of past selection pressures or have evolved via sensory bias or runaway selection, only persisting because of negligible maintenance costs (unreliable message signalling).

A recent review concluded that multiple message signalling is the most common mechanism for the maintenance of multiple signals in birds (Candolin 2003). This seems intuitively likely, as signallers should benefit from maximising the information available that can be assessed by receivers (Johnstone 1995). Nevertheless, the functions and intended receivers of individual display traits remain poorly understood for most
multicomponent displays. For example, since acoustic and visual ornaments are seldom quantified concurrently, little is known about potential interactions between these two categories of signals. This is of particular interest because acoustic and visual signals are strikingly different in their effectiveness over large distances. Acoustic signals can be assessed at both long and short distances from the signaller, yet visual ornaments can only be detected at close range. Thus conspecifics may assess males via a hierarchical process, initially prioritising long-range ornaments and then switching to visual ornaments when at closer distances to the signaller (e.g. Andersson 1989). Additionally, most studies typically quantify a limited range of male qualities, despite theory suggesting that individual ornaments may advertise different aspects of male condition (Johnstone 1995). In particular, many researchers fail to consider the selective pressures of extra-pair mating opportunities on signal evolution. Sexual cues used to assess social mate quality may differ from cues used in extra-pair mate choice (e.g. Catchpole 1986; Bensch and Hasselquist 1992). Thus, comprehensive studies concurrently quantifying a large range of male qualities and both acoustic and visual ornaments are desirable.

Here I investigate how the multicomponent displays of golden whistlers function in advertising various components of male phenotypic quality. Males display a range of colourful plumage patches including carotenoid-based yellow breast and nape plumage, a black chin stripe and a white throat patch. In addition, males are highly vociferous and possess large song repertoires (Chapter 3; van Dongen in press). I quantify a range of visual and acoustic ornaments and male qualities, including reproductive output, paternal care and territory size to explore the signalling capabilities of each ornament and the extent to which the expression of each ornament is shaped by male and female selection.

Methods

Study site and species

This study was carried out between September 2001 and February 2004 at Toolangi State Forest, Victoria, Australia (37°31' S, 145° 32'E). The study area initially covered 80 ha from a continuous stretch of forest covering approximately 38 000 ha (2001), but I
expanded this to 106 ha in September 2002. The predominant vegetation is a mountain ash
(\textit{Eucalyptus regnans}) canopy, with a variable understorey dependent on local topography
(Chapter 2; van Dongen and Yocom 2005).

The golden whistler is a socially monogamous passerine. Males attain full adult plumage in
their third year, but these males can often be distinguished from older males by traces of
juvenile plumage. The study population is migratory. Individuals arrive in early to mid-
September and depart in late April. Both sexes within a pair vigorously defend their
territory throughout this time.

\textit{Capture methods and morphometric measures}

Individuals were captured upon arrival at the study site or at the onset of breeding. Birds
were caught in mist nets and fitted with a unique combination of three coloured leg rings.
The approximate age of adult males was estimated based on the presence (first year adult
males) or absence (all other adult males) of traces of juvenile plumage. I made standard
measurements including head-bill length (distance from the tip of the beak to the back of
the head, to the nearest 0.1 mm, using dial callipers), tarsus length (nearest 0.1 mm,
callipers) and body mass (nearest 0.1 g, spring balance). The length and width (in mm) of
the elliptical throat patch was measured using dial callipers ensuring the male’s head was
fully outstretched to minimise variation in patch size due to the angle of the head relative to
the body. Throat patch area was then calculated using the following equation: throat patch
area = \pi \times (\text{width}/2) \times (\text{length}/2). Average chin-stripe width was calculated by averaging
three width measurements taken from the left, middle and right sides of the chin-stripe.
Nape-band width was measured in the same way. I also took a small blood sample (50 µl)
from the brachial vein of each bird for subsequent genetic analysis.

\textit{Objective quantification of plumage colour}

In 2001 and 2002, spectral reflectance properties of the yellow breast and white throat
patch were measured using a S2000 spectrometer, PX-2 pulse xenon light source, a fibre-
optic reflectance probe and OOIBase32 software (Ocean Optics, Inc., Dunedin, USA). Before the measurement of each bird the spectrophotometer was calibrated against a Spectralon white (Labsphere Inc.) and dark reference. This was repeated after each plumage region was measured. I took four replicate measurements from each plumage region each bird, placing the probe with its machined 45° angle end flat against the feathers. Reflectance measurements were taken at 3 nm increments from 300 to 700 nm.

The multiple spectral curves of each plumage region were averaged and used to derive three colour variables: plumage brightness, hue and chroma. Plumage brightness (spectral intensity) was estimated from the sum of reflectances between 300 and 700 nm \( R_{300-700} \). Plumage hue (spectral location or ‘yellowness’) was estimated from \( \lambda(R_{50}) \): the midpoint wavelength between the wavelength of maximum \( R_{\max} \) and minimum \( R_{\min} \) reflectances. Plumage chroma (spectral saturation) was estimated by dividing the reflectance between the wavelengths at which yellow reflects maximally (450 – 700 nm) by total reflectance \( R_{450-700}/R_{\text{Total}} \). These measures of colour were highly repeatable for the four replicates taken from each plumage region (brightness: \( r^2 = 0.509, F_{1,36} = 12.58, p = 0.001 \); chroma: \( r^2 = 0.440, F_{1,36} = 7.68, p = 0.009 \); hue: \( r^2 = 0.686, F_{1,36} = 31.11, p < 0.001 \)).

**Daily census and nest monitoring**

The population was monitored via daily censuses throughout the breeding season. I monitored 25 pairs in 2001, 27 in 2002 and 24 in 2003. After locating an individual, I noted its position in relation to a set of fixed reference points spread throughout the study site (97 reference points over 106 hectares). Male territories were mapped using the geographic information system software package ArcView GIS 3.2 (Environmental Systems Research Institute 1999). I mapped territories for males in 2001 and 2002. Territory sizes were calculated where I had a minimum of eight fixes per male from different days (mean number of fixes per male: 2001: 11.1 ± 3.0, \( n = 18 \), 2002: 12.4 ± 4.0, \( n = 20 \)).

Nests were located for each pair and monitored every two or three days. A blood sample was taken from nestlings for paternity analysis.
Behavioural observations

In 2001, recordings were made using a Sony TCD-D8 Digital Audio Tape recorder and Sennheiser ME67 unidirectional microphone. I opportunistically recorded song time budgets throughout the breeding season (mean song time budget duration: 21 min 19 secs ± 11 min 34 secs SD). In 2002, I documented individual variation in singing rates by following an individual for a 30-minute period and counting all songs sung. I performed these counts during four stages of breeding season: pre-breeding, nest building, egg incubation and post-breeding (see Chapter 3 for details). In 2003, recordings were made in conjunction with simulated territory intrusion experiments using live caged decoy birds and song broadcast from speakers (Chapter 7). I made recordings using a Sony TC-D5 pro-stereo cassette recorder and Sennheiser ME67 microphone. I was thus able to estimate male singing rates in 2001 and 2002 and male repertoire sizes in 2001 and 2003. Male song repertoires were determined by calculating the total number of different song-types sung by an individual male for all recordings in a given year. Repertoire exhaustion curves were constructed to ensure that the majority of song-types were logged for each male (Chapter 3; van Dongen in press).

I quantified parental effort at the nest by conducting nest watches during the incubation period during 2001. Males usually did not assist in incubation until 5 days after the complete clutch had been laid, so all watches occurred after this time (Chapter 2; van Dongen and Yocom 2005). Incubation observations were 90 min in duration, during which time I was hidden from view at a distance of 8 – 12 m from the nest. During the observation, I recorded the number of incubation bouts and the duration of each incubation bout for each sex.

Nestling paternity assessment using polymorphic microsatellites

DNA was extracted from all blood samples using a salting out procedure (Bruford et al. 1992) and stored in TE buffer (10 mM Tris pH7.5, 0.1 mM EDTA). All nestlings and adults were genotyped at 6 loci (Ppm1, 3, 7, 8, 10 and 11) using procedures described in
Chapter 4. These six loci permitted a high probability of unambiguously identifying the genetic father of each nestling (exclusion probability = 0.9997).

To determine the genetic father of each nestling ($n = 130$) I first established whether the social father was also the genetic sire of that nestling. The genotype of nestling’s sire was determined by subtracting the maternal alleles from the nestling’s genotype. I then compared the genotype of the putative sire to that of the social father. Nestlings that matched their social father’s genotype at all loci were considered within-pair ($n = 80$). One-allele mismatches between social father and offspring occurred in 25 cases. I conservatively assumed these were mutations or typing errors because all other loci matched the nestling to the social father and no other males in the population provided a better match. I assigned nestlings as extra-pair in cases where nestling genotypes differed at more than one locus from that of the social father ($n = 25$). To determine the genetic father of these nestlings, I searched a database of all genotyped males present in that year for individuals that possessed all the paternal alleles of the nestling. In five cases, one-allele mismatches occurred between the assigned genetic father and offspring. This was again assumed to be a mutation or typing error, because no other males provided a better match. However, no male genotypes within the database matched the paternal alleles of an additional five nestlings. These individuals were presumably sired by unbanded extra-pair males. In all cases where I assigned a father ($n = 125$), I was confident in my assignment of paternity because only one male matched the non-maternal genotype of nestlings at at least five loci. Paternity assignment was later confirmed using the maximum likelihood procedure in the computer programme CERVUS (Marshall 2001), setting the minimum confidence level of correctly assigning paternity to 80%. In all cases, the most likely fathers as determined from the exclusion method were identical to those nominated by CERVUS.

**Statistical analysis**

All data were tested for normality and transformed where necessary. I used General Linear Models (GLM) or Generalised Linear Mixed Models (GLMM) depending on the nature of the data. GLMs were used for data that were only collected for one season. My data on
male reproductive success were potentially non-independent due to males re-nesting several times within a year or being present for more than one breeding season. I therefore utilised GLMMs, incorporating male identity as a random factor. Models were produced by entering all variables into the full model and dropping non-significant factors and interactions in order of increasing significance (i.e. from those factors that were highly non-significant to those that were only marginally non-significant). Thus only significant variables remained in the model. I then assessed the excluded variables for their lack of significant contribution to the model by re-entering them one by one. The model containing all the significant factors and interactions was then termed the ‘final model’.

Several measures were used to estimate male reproductive success within a season: the number of within-pair young (WPY) sired, the number of extra-pair young (EPY) sired, and the total number of offspring sired (WPY + EPY). Within-pair success was firstly quantified via a binomial test of whether or not a male was cuckolded during that breeding season. I then quantified the proportion of extra-pair nestlings in all the males’ nests. Extra-pair success was measured using both a binomial test (whether a male gained extra-pair fertilisations within a single season) and the total number of extra-pair young sired within that season. Total reproductive success was estimated using both the total number of young sired, and the total number of nestlings sired by a male that fledged in a given season.

In all analyses involving male song repertoire size I included male age in the model, as older males are known to possess larger song repertoires (Chapter 3; van Dongen in press). In analyses involving multiple tests I conducted Bonferroni corrections. To reduce the subsequent risk of type II errors, I increased the alpha level to 0.1 for these tests only (Wright 1992; Chandler 1995). All GLMs were analysed using SPSS 12.0 (SPSS Inc, Chicago, Illinois, USA) and all GLMMs using Genstat 7.0 (Lawes Agricultural Trust, 2003). Data are reported as means and standard deviations unless otherwise stated.
Results

Inter-correlations between male ornaments

Throat patch size was positively related to both throat patch brightness and breast brightness, while males with brighter throat patches also possessed ‘yellower’ breast plumage (i.e. higher hue values; Table 1). In addition, males possessing larger song repertoires displayed wider chin stripes. Finally, breast chroma was negatively related to breast brightness. However, all significant correlations became non-significant after Bonferroni corrections were applied (Bonferroni-corrected critical value for 36 correlations is \( p = 0.003 \)).

Male ornaments and male-male competition

Males possessing large throat patches defended larger territories (\( r^2 = 0.72, F_{1,11} = 25.55, p < 0.001 \); Fig. 1), as did males singing at high rates (mean song rate across four breeding stages: \( r^2 = 0.30, F_{1,12} = 4.69, p = 0.053 \); mean song rate across nest building and incubation: \( r^2 = 0.33, F_{1,12} = 5.34, p = 0.041 \)). A synergistic interaction between song rate and throat patch existed, with the effect of singing rates on territory size being greatest for males with large throat patches and vice versa (overall model: \( F_{3,5} = 24.02, p = 0.002 \); throat patch size: \( F_{1,5} = 4.44, p = 0.089 \); song rate: \( F_{1,5} = 9.09, p = 0.030 \); throat patch*song rate interaction: \( F_{1,5} = 10.43, p = 0.023 \)).

Males with larger throat patches commenced breeding earlier (effect: \(-0.111 \pm 0.04; F_{1,15} = 6.39, p = 0.024\)). This did not result in a higher annual fledgling success for those males (\( F_{1,41} = 0.002, p = 0.967 \)), but males that commenced breeding earlier sired more young in a season (effect = \(-0.03 \pm 0.01, \text{Wald} = 4.25, n = 16, p = 0.039 \)). The size of the throat patch was unrelated to either male age or body size (male age: \( F_{1,19} = 0.452, p = 0.509 \); male body size [tarsus length]: \( F_{1,19} = 0.131, p = 0.721 \)).
Male ornaments and female attraction

Male incubation effort

No display signals predicted male incubation effort. However, males with smaller tarsi incubated for a greater proportion of time (effect = -0.07 ± 0.03, $F_{1,17} = 5.26, p = 0.036$), as did males defending smaller territories (effect = -3.77 ± 1.78, $F_{1,17} = 4.48, p = 0.05$).

Golden whistler mating system

Overall, 19.2% of all offspring genotyped were sired by a male other than the social father and 23.1% of all nests contained at least one extra-pair young. Of the 65 genotyped broods, only seven contained nestlings of mixed paternity. Eight nests contained only extra-pair nestlings, while the remaining 50 contained only nestlings sired by the social father. Of the eight nests that had more than one extra-pair nestling, only one contained nestlings sired by more than one extra-pair male. Eighty percent (16/20) of extra-pair nestlings with known fathers were sired by males defending territories immediately adjacent to that of the social father, fifteen percent (3/20) by a male two territories away, while one nestling was sired by a male three territories away.

Male reproductive success

Within-pair success

Males possessing large song repertoire sizes were less likely to be cuckolded (Table 2). This relationship persisted even after controlling for male age. Males gaining extra-pair fertilisations tended to have larger song repertoires than the males they cuckolded (EP mate repertoire: 11.3 ± 1.0 SD song-types, cuckolded mate repertoire: 5.8 ± 3.0 SD song-types; paired t-test, $t=-2.55, n = 4, p=0.084$). In addition, these cuckolding males also possessed ‘yellower’ plumage than the males they cuckolded (EP mate breast hue: 568 ± 3 nm, cuckolded mate breast hue: 560 ± 6 nm; paired t-test, $t = -2.98, n = 4, p = 0.059$).
Males possessing large song repertoires sired more young within their nest as did males with breast plumage that was duller and had higher values of hue and chroma (Table 2).

Extra-pair success

Males singing at high rates were less successful in attracting extra-pair fertilisations (Table 2). I could not determine whether song rates during each breeding stage could explain more variation in the number of extra-pair young sired due to the small sample of males with known singing rates during each stage who also sired extra-pair young.

Total reproductive success

Males that sang more during the nest building stage sired fewer offspring (Table 2; Fig. 2a). In addition, males with larger song repertoires and ‘yellower’ breast plumage had higher reproductive success (Table 2; Fig. 2b and 2c, respectively). Males possessing larger song repertoires and ‘yellower’ breast plumage (i.e. higher hue values) were also more successful in producing fledglings (song repertoire: effect = 0.18 ± 0.08, n = 28, Wald = 5.45, p = 0.02; breast hue: effect = 0.063 ± 0.032, n = 28, Wald = 3.89, p = 0.049; Table 2). Males that successfully raised offspring to fledging within a season possessed ‘yellower’ plumage than unsuccessful males (male breast hue – successful: 568 ± 2 nm, unsuccessful: 560 ± 3 nm, Wald = 4.33, n = 21, p = 0.037), and tended to have larger repertoires (male song repertoire – successful: 11.3 ± 0.6 song-types, unsuccessful: 8.5 ± 1.3 song-types, Wald = 2.90, n = 21, p = 0.089).

Discussion

The conspicuous visual and acoustic ornaments of male golden whistlers may function as intra- or inter-sexual signals, and may furthermore convey information to conspecifics at varying distances. Plumage signals, for example, typically function in short-range communication, as these visual ornaments cannot readily be assessed at large distances. In contrast, song is an efficient means to convey information to conspecifics at longer distances. Additionally, plumage and behavioural ornaments vary greatly in plasticity and
thus capability of reflecting long- or short-term male quality. In the golden whistler, singing rates and throat patch size appear to function as long- and short-range signals respectively, and are predominantly used in male-male interactions. In contrast, females may assess song repertoire size of potential mates at long distances and breast colour during close-range encounters. Each ornament thus appears to convey information about different aspects of male quality to different receivers at variable distances. There was no intercorrelation between the expression of the various display traits. Thus, multiple ornaments in this species appear to have evolved primarily via multiple message mechanisms.

Short-range signals

Intrasexual interactions

Male signals of social status are common across a diverse range of taxa including birds, reptiles and insects (Pryke and Andersson 2003; Whiting et al. 2003; Tibbetts and Dale 2004). In birds, males expressing an enhanced signal have often been shown to possess superior competitive abilities and be able to secure better resources, such as larger territories or the monopoly of limited food resources (e.g. Pryke and Andersson 2003; Evans 2004; Ferns and Hinsley 2004). Likewise, the throat patch size of male golden whistlers appears to advertise dominance during close-range male disputes. Individuals with larger patches defended larger territories and, during simulated territory intrusions, males with larger patches received more aggressive attacks from conspecific males but not females (Chapter 6, 7). During disputes, golden whistlers positioned themselves upon adjacent vegetation and postured by flattening their bodies dorso-ventrally and cocking back their heads to prominently display their throat patches. Before engaging in such contests, individuals appear to assess the relative throat patch size of a rival, since resident males respond quicker to intruders with similar sized patches (Chapter 6). Males initially assessing the competitive abilities of rivals using status signals has been reported in many species (Balph et al. 1979; Møller 1987; Pryke et al. 2002) and may settle disputes between highly mismatched individuals without the high costs associated with fighting (Rohwer 1982).
Male whistlers with large throat patches also commenced breeding earlier, supporting the idea that males with large patches are of higher quality. Laying date has been consistently shown to be a reliable measure of quality in many species and early nesting pairs typically enjoy an increased reproductive success, even after controlling for individual quality (Verhulst et al. 1995; Sanz 1999; Hasselquist et al. 2001). However, it is unknown whether the association between patch size and laying date is due to male whistlers with larger patches arriving earlier and being able to initiate breeding activities sooner (e.g. Cristol 1995; Béty et al. 2003). This is because poor weather conditions and unobtrusive behaviour by the males upon arrival at the breeding site prevented arrival dates from being accurately estimated. Alternatively, males with large patches may commence breeding earlier for reasons unrelated to arrival date and be due to factors related to the higher quality of these individuals (e.g. Hasselquist et al. 2001).

Intersexual interactions

The development and maintenance of elaborate plumage displays typically entails high costs for the signaller, resulting in associations between plumage expression and male phenotypic or genetic quality (e.g. Hill 1999; Evans 2004). Females are therefore expected to use these traits when assessing males as social or genetic partners at close range. Increasing studies suggest that males with more elaborate ornaments are of high genetic or phenotypic quality and enjoy a greater reproductive success than less well-adorned individuals (e.g. Yezerinac and Weatherhead 1997; Møller et al. 1998; McGraw et al. 2001; Pryke et al. 2001). In golden whistlers, male breast plumage colour also reliably predicted reproductive success. Males with duller plumage were more likely to be cuckolded and males gaining extra-pair fertilisations possessed ‘yellower’ plumage (i.e. higher hue value) than the males they cuckolded. Breast hue was also positively associated with the total number of offspring sired and the pair’s fledging success.

The associations between breast colour and reproductive success suggest that females gain information regarding male quality by assessing this ornament during social and extra-pair mate choice decisions. The yellow colouration of the plumage is primarily due to the presence of the carotenoid lutein in the feathers (High Performance Liquid
Chromatography analysis; S. Andersson, pers. comm.). Although carotenoids are abundant in plants, they cannot be synthesised *de novo* by animals and so must be extracted from the birds’ diet (Goodwin 1976; Goodwin 1984). The carotenoid content of a male’s plumage may therefore reliably reflect foraging abilities or access to high quality food resources (Hill 1990). Since carotenoids are required for efficient immune function and are therefore essential to maintain the condition of the signaller (Lozano 1994; Hill 1999) the amount of carotenoids allocated to plumage pigmentation may be highest among high quality individuals. It is currently unknown whether lutein is a limited resource in the golden whistler diet. However, this pigment is typically the most abundant in nature (Goodwin 1976). Thus, although there may be some production costs associated with the yellow plumage, maintenance, social or ecological costs may be more relevant. Regardless of the exact costs incurred, this ornament still appears to be a vital component of female mate choice decisions.

*Long-range signals*

Intrasexual interactions

Singing permits efficient long-distance communication between conspecifics. Much information may be encoded in song structural features and singing behaviour of males (Catchpole and Slater 1995; ten Cate *et al.* 2002). The structural aspects of song are typically important for intersexual communication (e.g. Hasselquist *et al.* 1996). On the other hand, dynamic song traits, such as singing rates, are often used as antagonistic signals during male competition (Catchpole and Slater 1995). Male golden whistlers singing at high rates defended larger territories, suggesting that song functions as a long-range antagonistic signal, as it does in many other species (e.g. Naguib 1999; Hall 2000; Peake *et al.* 2001; Mennill and Ratcliffe 2004). However, variation in song rates between different breeding stages suggests an additional, more complex function of singing rates. Song output is low upon arrival at the breeding site but increases dramatically when breeding commences (Chapter 3; van Dongen *in press*). In other species, this increase in singing output is hypothesised to allow individuals to guard their brood from potential cuckolders and attract extra-pair matings from neighbouring females (Møller 1991b). According to this
hypothesis, males announce the fertility of their female by increasing song features related to quality to advertise their quality to neighbouring individuals (Slagsvold et al. 1994; Welling et al. 1995; Ballentine et al. 2003). Males with high song output presumably have superior competitive abilities and may be paired to females less willing to accept extra-pair matings from other individuals. Potential cuckolders may thus selectively pursue extra-pair fertilisations with females paired to males with low song output.

Golden whistler singing rates during the female fertile period were unrelated to within-pair reproductive success. However, males broadcasting song at high rates during this period sire fewer total offspring. Due to the low number of males with known singing rates during the fertile period that also sired extra-pair offspring, I could not assess whether this pattern was a result of differences in extra-pair success. Nevertheless, this seems likely as singing rates were unrelated to within-pair reproductive success. This raises the interesting possibility of condition-dependent signalling strategies whereby males that are more likely to be cuckolded benefit from investing more resources into brood guarding via singing at the expense of pursuing extra-pair matings (Kokko and Morrell 2005). Mate-guarding and extra-pair forays have been shown to be mutually exclusive activities (Chuang-Dobbs et al. 2001) and, in golden whistlers, this is supported by the fact that singing rates during the fertile period are lowest when a high number of neighbouring females are receptive. This relationship disappears once mate-guarding is no longer necessary (Chapter 3; van Dongen in press). Low quality males may thus enjoy the same within-brood reproductive success as more attractive males, but sire less extra-pair young. Yet, no negative associations exist between male ‘attractiveness’ (e.g. breast hue) and singing rates during the fertile period. This raises the possibility that an, as yet unmeasured, phenotypic trait correlates negatively with song rate that is additionally used to assess extra-pair mates. Alternatively, males may increase singing rates during breeding for reasons independent of mate guarding, such as territory defence. Due to high intrapopulation variation in male attractiveness and competitive abilities, some males may benefit from investing more into territory defence to gain better resources at the expense of pursuing extra-pair matings. Tradeoffs between mate attraction and competitive behaviour have been documented in other species (e.g. house finches, *Carpodacus mexicanus*; Duckworth et al. 2004).
After the fertile period, males continued to sing at high rates, although song output during this period was unrelated to male reproductive success. Singing during this period may instead advertise male presence to neighbouring females, after which other cues are used to assess extra-pair mates (e.g. song repertoire size). For example, in the lekking Jackson’s widowbird (*Euplectes jacksoni*), male display rate determines the number of female visits to leks, after which potential mates are assessed based on tail length (Andersson 1989).

High singing rates may also reflect a greater need for territory defence during this period. It is thought that nestling provisioning is costly due to high energy demands (Bryant 1988; Bryant and Tatner 1991). This may require greater access to food sources which should thus be defended more intensely. Since singing rates are positively related to territory size and all foraging activities occur exclusively within each individual’s territory, increasing singing rates to increase territory size would lead to access to more food resources.

**Intersexual interactions**

Males with larger song repertoire sizes enjoyed greater reproductive success. They were less likely to be cuckolded, sired more offspring and raised more young to fledging. Males that gained extra-pair fertilisations possessed larger song repertoires than the male they cuckolded, suggesting that females not only used song repertoires in decisions on whether to seek extra-pair matings, but also selected extra-pair males based on repertoire size. In many species, variation in repertoire size reflects individual genetic quality (Hasselquist et al. 1996) or past developmental stresses (Nowicki et al. 1998; Spencer et al. 2003). In golden whistlers, song repertoire size is unrelated to any phenotypic traits such as territory size or male incubation effort. Therefore, females may mate with these males solely for genetic benefits, although the possibility that song repertoire size also advertises some other, as yet unmeasured, male phenotypic quality still remains. In addition, repertoire size appears to be, in part, related to male age (Chapter 3; van Dongen *in press*). This pattern is common in many species and may arise if older males have a larger time frame in which to augment their repertoires (e.g. Hasselquist *et al.* 1996; Gil *et al.* 2001). Females may thus be selecting for older and more experienced males instead of genetically superior males. Yet, after statistically controlling for age, golden whistlers with large repertoires still
enjoyed high reproductive success, suggesting that females gain additional information from assessing song repertoires, independent of age.

Why do males display several potentially costly ornaments?

The existence of multiple, potentially costly ornaments in golden whistler displays appears to have evolved primarily via multiple message mechanisms (Møller and Pomiankowski 1993). Different ornaments are directed towards different receivers, signal different qualities and convey information at different distances. Using single ornaments to advertise each particular aspect of male quality may increase the efficiency via which information can be broadcast to conspecifics by reducing production costs via the expression of several costly ornaments reflecting the same quality. Most other studies investigating multiple ornaments in birds have similarly found evidence for a multiple signalling function (Candolin 2003). Thus, there is growing evidence that this may be the most widespread explanation for multicomponent displays. In golden whistlers, song rate and repertoire, throat patch size and breast colour appear to be the most important components of whistler displays, while the nape band, chin stripe and brightness of the throat patch seem not to be under strong selection. Since there was no inter-correlation between the expression of the four principal display ornaments, conspecifics presumably assess each independently to obtain information on different aspects of male quality. Song rate appears to be used as a long-range signal during male interactions and, as a highly plastic trait, may reflect the signaller’s short-term condition. Throat patch size appears to be used in close-range male encounters and may reflect long-term viability or competitive abilities. Song repertoire size and breast colour appear to be used by females to assess both extra-pair and social mates. This is in contrast to other species in which different cues are typically used during social and extra-pair mate choice decisions (e.g. great reed warblers, *Acrocephalus arundinaceus*; Catchpole 1986; Bensch and Hasselquist 1992) and suggests that females gain both direct and indirect benefits from mating with these males. Yet, the lack of any interaction between the two ornaments in predicting reproductive success implies that females assess the two traits separately to gain information about different aspects of male quality. Additionally, differences in the effectiveness of the two signals over large distances may also mean that females select males via a hierarchical process, listening to song repertoires
while still in their territory and then visiting a subset of preferred males to assessing breast
colour in close proximity. Breast plumage quality and song repertoire size may be preferred
signals for females because they are static traits that reflect male viability more reliably
than other signals such as singing rates (e.g. Møller et al. 1998).

I found some evidence for unreliable message signalling. Although the chin-stripe and nape
band are both conspicuous plumage patches, they did not predict any aspect of male
quality. Yet, while these ornaments may thus be vestiges of past selection pressures,
alternate explanations are more likely. The chin-stripe may have no explicit signalling
function, but may instead augment the contrast around the throat patch, increasing its
visibility during assessment by conspecifics (e.g. Mennill et al. 2003). I was unable to
quantify colour variation in the nape band, as this band is interspersed with variable
numbers of olive feathers. However, if equal amounts of carotenoids are deposited into the
breast and nape plumage, then the colour attributes of these ornaments will be inter-
correlated. Females could therefore assess variation in yellow plumage using both breast
and nape band colour. Finally, though various ornamental traits were positively inter-
correlated, I did not find evidence that they signalled similar aspects of male quality. Thus I
found no evidence supporting backup message signalling.
Table 1. Pearson correlation matrix for male golden whistler ornamental traits measured within a single season. Values are Pearson correlation values (r) and asterisks denote significance at the 0.05 level. All significant correlations became non-significant after Bonferroni corrections were applied (Bonferroni-corrected critical value for 36 correlations is $p = 0.003$).

<table>
<thead>
<tr>
<th></th>
<th>Throat Patch Area</th>
<th>Throat Patch Brightness</th>
<th>Chin Stripe Width</th>
<th>Nape Band Width</th>
<th>Total Song Rate</th>
<th>Song Repertoire Size</th>
<th>Breast Brightness</th>
<th>Breast Hue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat patch brightness</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Chin stripe width</td>
<td>-0.096</td>
<td>0.024</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nape band width</td>
<td>-0.373</td>
<td>0.081</td>
<td>0.054</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total song rate</td>
<td>0.253</td>
<td>0.046</td>
<td>0.132</td>
<td>-0.498</td>
<td></td>
<td></td>
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<tr>
<td>Song repertoire size</td>
<td>-0.600</td>
<td>0.050</td>
<td>0.426*</td>
<td>0.068</td>
<td>0.289</td>
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<tr>
<td>Breast brightness</td>
<td>0.626*</td>
<td>0.086</td>
<td>0.246</td>
<td>0.133</td>
<td>0.253</td>
<td>0.476</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast hue</td>
<td>0.309</td>
<td>0.364*</td>
<td>0.007</td>
<td>-0.187</td>
<td>-0.099</td>
<td>-0.079</td>
<td>-0.036</td>
<td></td>
</tr>
<tr>
<td>Breast chroma</td>
<td>-0.111</td>
<td>0.078</td>
<td>-0.063</td>
<td>-0.293</td>
<td>0.091</td>
<td>0.110</td>
<td>-0.335*</td>
<td>0.014</td>
</tr>
</tbody>
</table>
Table 2. GLMM models for the effects of male traits on reproductive success. Only variables with p < 0.1 are reported for presentation purposes. Variables with asterisks were included in the final model and are presented using the final model statistics. Variables without asterisks were significant, but were omitted from the final model. In all models incorporating song repertoire size, I also controlled for male age. 

a) Probability of being cuckolded; binomial model with logit link. 0 = not cuckolded, 1 = cuckolded. 
b) Proportion of extra-pair young in nest; Poisson model with logarithm link. Note that song repertoire was highly significant but could not be included in the final model because of the low number of males that had both plumage colour and song repertoire quantified. 
c) Number of extra-pair young sired; Poisson model with logarithm link. Note that song rate during each breeding stage could not be analysed due to low numbers of males with known song rates during each stage that also sired extra-pair young. 
d) Total offspring sired. normal distribution with identity link. 
e) Number of offspring that fledged; Poisson model with logarithm link.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect</th>
<th>Standard Error</th>
<th>Wald Statistic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a) Probability of being cuckolded</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Constant*</td>
<td>-1.20</td>
<td>1.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Song Repertoire size*</td>
<td>-0.48</td>
<td>0.25</td>
<td>3.70</td>
<td>0.054</td>
</tr>
<tr>
<td><strong>b) Proportion of extra-pair young in nest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Song Repertoire Size</td>
<td>-2.18</td>
<td>0.11</td>
<td>5.55</td>
<td>0.018</td>
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<tr>
<td>Constant*</td>
<td>-6.10</td>
<td>0.70</td>
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<td>Breast Brightness*</td>
<td>0.0006</td>
<td>0.0002</td>
<td>10.66</td>
<td>0.001</td>
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<tr>
<td>Breast Chroma*</td>
<td>-10.51</td>
<td>2.73</td>
<td>14.73</td>
<td>&lt;0.001</td>
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<tr>
<td>Breast Hue*</td>
<td>-0.02</td>
<td>0.003</td>
<td>79.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>c) Number of extra-pair young sired</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant*</td>
<td>-1.34</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Song rate*</td>
<td>-0.35</td>
<td>0.14</td>
<td>6.61</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>d) Total offspring sired</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nest Building Song Rate</td>
<td>-0.64</td>
<td>0.24</td>
<td>7.17</td>
<td>0.007</td>
</tr>
<tr>
<td>Song Repertoire Size</td>
<td>0.20</td>
<td>0.08</td>
<td>6.03</td>
<td>0.014</td>
</tr>
<tr>
<td>Constant*</td>
<td>2.12</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast hue*</td>
<td>0.09</td>
<td>0.03</td>
<td>8.18</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>e) Number of offspring fledged</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast hue</td>
<td>0.06</td>
<td>0.03</td>
<td>3.89</td>
<td>0.049</td>
</tr>
<tr>
<td>Constant*</td>
<td>-0.78</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Song Repertoire Size*</td>
<td>0.18</td>
<td>0.08</td>
<td>5.45</td>
<td>0.02</td>
</tr>
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</table>
Figure 1. The size of the territory defended by a male golden whistler and the size of his throat patch. Open circles represent males singing at low rate (less than the mean rate of 5.7 songs/min) and closed circles are males singing at high rates (more than 5.7 songs/min). Note that the final model includes an interaction between song rate and throat patch size such that the effect of throat patch on territory size is greatest for males singing at the highest rates and vice versa (overall model: $F_{3,5} = 24.02, p = 0.002$; throat patch size: $F_{1,5} = 4.44, p = 0.089$; song rate: $F_{1,5} = 9.09, p = 0.030$; throat patch*song rate interaction: $F_{1,5} = 10.43, p = 0.023$).
Figure 2a. The total number of young sired by male golden whistlers within a season (within-pair young and extra-pair young) and song rate during the nest building stage (effect = -0.64 ± 0.24, Wald = 7.17, p = 0.007).
Figure 2b. The total number of young sired by male golden whistlers within a season (within-pair young and extra-pair young) and song repertoire size (effect = 0.20 ± 0.08, Wald = 6.03, p = 0.014).
**Figure 2c.** The total number of young sired by male golden whistlers within a season (within-pair young and extra-pair young) and breast hue (effect = 0.09 ± 0.03, Wald = 8.18, p = 0.004).
Chapter 6

The throat patch and male-male aggression in golden whistlers
(*Pachycephala pectoralis*)

Unpublished manuscript

Abstract

In many birds, male antagonistic signals advertise competitive abilities to conspecifics and individuals with larger signals typically have access to higher quality resources. However, associations between status signals and dominance may be misleading as such signals are often inter-correlated with other aspects of male quality such as age or body size. Therefore, the experimental manipulation of such traits is crucial for a clearer understanding of the signal’s function. I experimentally investigated the role of a conspicuous white throat patch displayed by male golden whistlers (*Pachycephala pectoralis*) during male-male territorial disputes. Males with larger throat patches defend larger territories and commence breeding earlier. I manipulated the size of the throat patch of decoy caged males and presented them to both males and females in field-based simulated territorial intrusions. Females did not discriminate between ‘reduced’ throat patch or ‘control’ treatments. However, males displayed lower levels of aggression to caged males with smaller throat patches. In addition, focal males with throat patches similar in size to those of decoy males responded faster to the apparent threat. Thus, the white throat patch of male golden whistlers appears to be a signal of dominance among males.

Introduction

Conspicuous sexual displays are common in animals, honestly conveying information about the signallers’ phenotypic or genetic quality (Andersson 1994). Male ornaments may be used by females as cues during mate choice decisions or as signals of dominance or
social status during male-male encounters. Signals of dominance are often reliable predictors of outcomes during elevated male conflicts and can settle disputes between highly mismatched individuals without the high costs associated with fighting (Bradbury and Vehrencamp 1998; Senar 1999). As a consequence, males possessing higher quality status signals typically secure better resources, such as larger territories, access to feeding stations less exposed to predators or the monopoly of limited food resources (e.g. Evans and Hatchwell 1992; Pryke and Andersson 2003; Ferns and Hinsley 2004).

The benefits of expressing status signals are balanced by high production or maintenance costs that prevent cheating among poor quality individuals. Plumage status signals commonly incorporate melanin-based colouration (e.g. black and white plumage; Jawor and Breitwisch 2003). Melanin-based ornaments are traditionally thought to incur insignificant production costs on the signaller. However, increasing evidence suggests this view to be inaccurate and that high physiological costs may be borne by individuals during the development of these ornaments (reviewed by Jawor and Breitwisch 2003). In addition, status signals may be under strong social control. Males with large ornaments often suffer more aggressive attacks from dominant conspecifics with superior competitive abilities (Senar 1999). This appears to occur because individuals are typically more likely to engage in contests with rivals with similar sized ornaments (e.g. Balph et al. 1979; Møller 1987; Pryke et al. 2002). The costs incurred from these contests are often greater among subordinates, thus discouraging these inferior individuals from developing dishonestly large ornaments (Veiga 1995). Yet, many studies have failed to find evidence of social control of signal cheating (Järvi et al. 1987; Slotow et al. 1993; Solberg and Ringsby 1997), which thus remains a debated issue.

Behavioural studies investigating the maintenance of status signals and the regulation of cheating often report correlational relationships between male signal size and access to resources (e.g. Senar et al. 1993; Hein et al. 2003). However, other traits such as age or body size may be inter-correlated with status signal size and may independently influence male competitive ability (e.g. Hagelin 2002). Experimental studies in which the trait of interest is manipulated under controlled conditions are therefore essential to unambiguously assign a trait as a signal of dominance. Previous experiments have
generally revealed that males with augmented signals enjoy higher social status, but may suffer high social or physiological costs (e.g. Evans and Hatchwell 1992; Veiga 1995; Pryke and Andersson 2003). Yet such studies are typically conducted in aviaries under unnatural conditions (e.g. Hagelin 2002), or on free-living individuals that are free to interact with conspecifics without control (e.g. Evans and Hatchwell 1992; Ferns and Hinsley 2004). Experiments conducted in natural settings under controlled conditions are rare.

Here I report a study of a field-based experiment investigating a potential signal of dominance in male golden whistlers (*Pachycephala pectoralis*). Males display elaborately coloured plumage including a white throat patch. Males with large throat patches defend larger territories and commence breeding earlier (Chapter 5). This suggests that this ornament may signal dominance during male disputes. I manipulated the size of the throat patch of live ‘decoy’ males and presented them to free-living males and females during simulated territorial intrusions. I expected that if the throat patch is indeed a signal of male dominance, males should display clear-cut differences in their response to intruders possessing different sized patches. Furthermore, I investigated whether males assess the relative throat patch size of the rival when engaging in a contest. I discuss the results in light of the throat patch being exclusively directed towards males during close-range disputes.

**Methods**

**The study species and site**

The golden whistler is a socially monogamous passerine. Males display bright plumage including a yellow breast and white throat patch. In contrast, females possess a uniform grey-brown plumage. Both sexes participate in joint territory defence, but territorial interactions are sex-specific and female-male contests never occur (Chapter 2; van Dongen and Yocom 2005). This study was carried out between November 2002 and January 2003 at Toolangi State Forest, Victoria, Australia (37°31’ S, 145° 32’E). Individuals were captured and individually marked with a unique combination of three colour bands.
Twenty-seven territory-holding pairs were monitored daily throughout the breeding season. All nests were located and monitored every two or three days to determine the breeding status of the pair.

Decoy male capture and manipulation

Males used as live decoys were captured within the same forest, but outside the study area (typically between 1 – 5 km from the study site). When not used in an experiment, males were housed in cages measuring 120 x 60 x 60 cm and provided with meal worms ad libitum. I randomly assigned males to one of two groups – ‘reduced throat patch’ males and ‘control’ males. Throat patch sizes were reduced using black non-toxic ‘Sharpie’ markers (Sanford, USA) to blacken the outer edges of the white throat patch to resemble the surrounding black plumage. Throat patch area was then calculated by measuring the length and width (in mm) of the patch using dial callipers and using the following equation: throat patch area = $\pi \times \frac{\text{width}}{2} \times \frac{\text{length}}{2}$. I attempted to reduce patch sizes to the minimum limit of natural variation (approximately 460 mm$^2$), but a high level of precision was difficult. Control males were subjected to similar treatment but I applied clear ‘Setasilk’ dye thinner (Pèbèo, France), so that the size of the throat patch remained the same. The mean throat patch size of the ‘reduced’ group was significantly smaller than that of the control group (control: 538.7 ± 26.3 SD mm$^2$; reduced: 322.5 ± 106.4 SD mm$^2$; $F_{1,7} = 15.36, p = 0.006$). The plumage manipulations did not involve an increase in signal size because all white paints and dyes tested did not convincingly match the spectral colouration of the throat patch. However, studies in which signal sizes are manipulated in only one direction are common and have been able to convincingly show that individuals perceive and change their behaviour relative to variation in the trait of interest (Hagelin 2002; Ferns and Hinsley 2004; Senar et al. 2005; Torres and Velando 2005).

Playback recordings

Song recordings for the playback tracks were compiled using a Sony TCD-D8 Digital Audio Tape recorder and Sennheiser ME67 unidirectional microphone. I recorded songs
from males approximately 15 km from the study site to prevent variation in responses due to familiarity with songs. Three separate 5-min playback tracks were made. Each track incorporated 4 song-types recorded from a single male in a continuous loop of 5 songs per minute. I randomised the tracks used in each experiment.

**Experimental setup**

All trials of the simulated territorial intrusions (STIs) were conducted during the nest building period of each pair between 0630 and 1100 hours AEST, the peak singing period for this species (pers. obs.). A trial consisted of locating an individual and counting all songs sung by the individual during a 5-minute pre-playback period. A wire cage (18 x 18 x 36cm) containing the live decoy male was then placed on a stand (height: 1.2 m) within 20 m of the focal individual (mean distance from focal individual: 11.2 ± 3.3 SD m). Reduced or control decoy males were chosen randomly for each presentation. The playback track was broadcast from a Sony CDX-L460X portable car stereo with Realistic 30W speakers placed adjacent to the cage. Songs were broadcast at a volume approximating the natural intensity of golden whistler song (50 dB at 10 m, Lutron SL-4001 sound level meter). The decoy male never sang during the trials. During the 5-minute playback period, two observers were present, but hidden from view. One observer described the individuals’ behaviour, which was then transcribed by the second individual. The following response attributes were recorded: a) initial distance from cage (m); b) latency (s; the time elapsed before the focal individual displayed an obvious response, such as movement towards the cage, to the STI); c) number of songs sung; d) time spent within 5 m from the cage (s); e) distance of closest approach to the cage (m); f) final distance from the cage at the completion of the trial (m); g) the total duration of the response (s). Each trial was followed by a 5-minute post-playback period in which all songs were again counted.
Statistical analysis

All data were tested for normality and transformed where necessary. I also tested for equality of error variances using Levene’s test. In cases where variances between the two treatment groups were significantly different, I used non-parametric Mann-Whitney U tests. In all other cases I employed Generalised Linear Models (GLM). Experiment-wide Type I error rates were adjusted using Bonferroni correction when multiple tests were conducted. However to reduce the subsequent increased risk of type II errors, I increased the alpha level to 0.1 for these tests only (Wright 1992; Chandler 1995). In analyses investigating whether focal male traits predicted aggression levels during the trial, I pooled data from both treatments \( n = 25 \), but included treatment as a co-variate. When analysing the song output data, I incorporated singing rates during the pre-playback period as a covariate to control for differences in baseline vocal activities before the trials (Hall 2000).

An inadvertent consequence of throat patch reduction was the concurrent enhancement of chin stripe width. Associations between the focal individual’s behaviour and treatment may therefore be due to variation in chin stripe width and not throat patch size. To separate these effects, I regressed resident responses against manipulated chin stripe width. In all cases, the chin stripe width of the caged male was unrelated to resident male and female response during the simulated intrusions \( (p > 0.05) \). All analyses were conducted using SPSS 12.0 (SPSS Inc, Chicago, Illinois, USA).

Results

Sex differences in responses

Focal individuals did not always respond to the simulated intrusions alone. In all trials involving female subjects \( (n = 24) \), the male partner also responded. In addition, female partners responded in 40% of trials targeting male subjects (six of thirteen ‘control’ trials
and four of twelve ‘reduced’ trials). However, males did not change any aspect of their behaviour in the presence of female partners (all p > 0.05).

Female typically responded to the simulated intrusion trials by quietly approaching the caged male. They did not differ in any responses to the two treatment groups (Table 1). In contrast, males typically responded with high aggression and were more aggressive in response to control males than those with reduced throat patches. When presented with a control male, focal males spent more time within 5 m of the cage ($F_{1,21} = 4.84$, $p = 0.039$; Fig. 1) and were closer to the cage at the end of the playback ($U = -2.27$, $n_{\text{control}} = 13$, $n_{\text{reduced}} = 12$, $p = 0.022$; Fig. 1). These males also responded for a longer period of time ($U = -2.08$, $n_{\text{control}} = 13$, $n_{\text{reduced}} = 12$, $p = 0.037$; Fig. 1). The differences became non-significant when Bonferroni corrections were applied (Bonferroni-corrected critical value for 6 correlations is $p = 0.016$). However, the small samples sizes involved and hence low power of detecting significance (mean power $= 0.36 \pm 0.26$ SD, range: 0.05 – 0.63) warrants caution in the interpretation of these Bonferroni – corrected results. Male responses did not differ between the two treatments in terms of latency (control: $10.3 \pm 5.4$ secs; reduced: $16.1 \pm 13.6$ secs; $U = -0.759$, $n_{\text{control}} = 13$, $n_{\text{reduced}} = 12$, $p = 0.456$), singing rates (control: $6.6 \pm 3.8$ songs/min; reduced: $7.6 \pm 4.4$ songs/min; overall model: $F_{2,22} = 1.00$, $p = 0.383$, pre-playback song rate: $F_{1,22} = 1.65$, $p = 0.212$, treatment: $F_{1,22} = 0.444$, $p = 0.512$) and distance of closest approach (control: $4.1 \pm 5.3$ m; reduced: $3.8 \pm 3.6$ m; $F_{1,22} = 0.032$, $p = 0.444$).

**Focal male traits and aggression**

When focal males were matched with decoy males with similar sized throat patches, they tended to be quicker to respond to the presence of the decoy male (throat difference: $F_{1,11} = 4.707$, $p = 0.053$; treatment: $F_{1,11} = 0.965$, $p = 0.347$, Fig. 2). In one case, a male did not respond to the introduced decoy male until 71 seconds after the trial. Although this case was a statistical outlier (Grubbs’ test: $G = 2.775$, $n = 14$, $p = 0.01$), the trend remains upon
this data point’s removal (throat difference: $F_{1,10} = 4.734, p = 0.055$; treatment: $F_{1,10} = 1.158, p = 0.307$).

**Discussion**

Male golden whistlers directed increased levels of aggression towards rival males with large throat patches, investing a larger amount of time and effort in an attempt to expel the apparent threat from their territories. In natural close-range male-male encounters, individuals perch on vegetation in close proximity of each other and perform a posture display by dorsoventrally flattening themselves and prominently displaying their throat patch (Chapter 2; van Dongen and Yocom 2005). During these encounters, males may assess the relative size of the rival’s patch to make decisions about whether to escalate the contest. I found that, in comparison to closely matched contests, residents that had larger throat patches than the decoy male tended to take up to 60 seconds longer to react to the threat. This suggests that males are initially less willing to engage in potentially costly fights with inferior rivals, unless the threat persists for an extended period. Residents did not subsequently show greater aggression to these individuals in terms of both temporal responses and their distance from the stimulus. Females, on the other hand, appeared not to discriminate between males possessing different sized patches. Although females participate in territorial defence, territorial interactions are sex-specific, so that females only engage in physical contests with other females (Chapter 2; van Dongen and Yocom 2005). Thus the information content of the male’s throat patch may be of little interest to females. The throat patch of males therefore appears to be a short-range signal of dominance directed towards same-sex conspecifics.

The lower levels of aggression directed towards males with smaller throat patches suggests that cheating among subordinates may be regulated via regular dominance testing of individuals expressing large throat patches. Social control of cheating has been reported in several other species including dark-eyed juncos, *Junco hyemalis*, (Balph *et al.* 1979), house sparrows, *Passer domesticus* (Møller 1987) and red-collared widowbirds, *Euplectes*...
ardens (Pryke et al. 2002; but see Järvi et al. 1987; Slotow et al. 1993; Solberg and Ringsby 1997 for negative or inconclusive results). In these species, costly fights are more likely between individuals with similar sized plumage patches. Subordinates with dishonestly large badges thus attract more aggressive attacks from dominant individuals with superior competitive abilities (Maynard Smith and Harper 1988). These frequent physical battles may incur high costs associated with increased energy expenditure, risk of injury and depredation (Whiting et al. 2003), costs and risks that are presumably higher among low quality males. For example, yearling house sparrows with experimentally enlarged patches showed increased mortality compared to older individuals, suggesting high mortality costs for cheaters (Veiga 1995).

In natural situations, aggressive physical interactions between neighbouring golden whistler males are common, during which males engage in frequent chasing and singing (pers. obs.). There is therefore ample opportunity for frequent testing of dominance in this species. Yet, the efficacy of social control as a means of preventing cheating will depend not only on the frequency and costs of testing, but also on the benefits of cheating (Slotow et al. 1993). For example, the benefits accrued by male golden whistlers by defending a large territory, such as access to more food resources, may be greater than the costs associated with intermittent attacks from neighbouring conspecifics. Cheating may also be prevented via high physiological costs associated with the production of enhanced signals (reviewed by Jawor and Breitwisch 2003). Elevated testosterone levels are generally required for the enhanced expression of antagonistic displays in birds (e.g. Duckworth et al. 2004; Mougeot et al. 2004). In addition, males with increased levels of testosterone display show augmented levels of aggression to conspecifics and are more successful in physical contests (e.g. De Ridder et al. 2000; Duckworth et al. 2004). However, elevated testosterone levels also incur strong immunosuppressive effects which can typically only be borne by high quality individuals (Folstad and Karter 1992).

My plumage manipulations reduced both the width and length of the throat patch. In all individuals, the throat patch commences at the base of the lower mandible and extends to a black chin stripe approximately 20 mm below the mandible. Thus throat patch length could only be reduced via the widening of the chin-stripe. It is therefore possible that males
displayed higher aggression to males with smaller chin stripes rather than larger patches, \textit{per se}. However, I found no association between the width of chin stripes of decoy males and the resident’s response during the simulated intrusion. This suggests that a decrease in throat patch length alone is insufficient for conspecifics to detect an enhancement in patch size and that patch width is equally important. Additionally, in natural situations, chin stripe size is not associated with any aspect of male quality (Chapter 5). In my experiments, it thus seems likely that males are responding solely to the size of the throat patch. The chin-stripe may instead increase the contrast between the white throat patch and yellow breast plumage, facilitating assessment of throat patch size.

I have shown here that the white throat patch of male golden whistlers is directed exclusively to other males during elevated territorial disputes. Individuals may assess the relative throat patch size of the rival to avoid costly attacks between highly mismatched individuals. Additionally, males displaying reduced patches suffer less intense attacks from conspecifics, possibly acting as a mechanism to prevent cheating amongst subordinates. This study thus experimentally confirms my correlative findings that the throat patch functions as a signal of dominance during close-range male-male disputes.
**Table 1.** Female differences in responses to simulated territory intrusions (STI) of decoy males with control and reduced throat patches. Response variable are: latency (s: time lapsed before individual responded to STI), song rate during the playback period (songs/min), time spent within 5 m from the cage (s), closest distance the individual approached the cage (m), final distance at the completion of the playback (m) and duration of response (s: difference between time of first and last response). Means are presented with standard deviations. GLM were used when variances were equal. When this assumption was not met, I used non-parametric Mann-Whitney U tests. These cases are marked by an asterisk and only the test statistics are presented. In all cases $n_{\text{control}} = n_{\text{reduced}} = 24.$

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Control</th>
<th>Reduced</th>
<th>$F_{\text{numerator df, denominator df}}$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency</td>
<td>77.0 ± 109.5</td>
<td>54.5 ± 87.6</td>
<td>0.309 $_{1,22}$</td>
<td>0.584</td>
</tr>
<tr>
<td>Song rate</td>
<td>0.5 ± 0.4</td>
<td>0.5 ± 0.4</td>
<td>0.026 $_{1,22}$</td>
<td>0.873</td>
</tr>
<tr>
<td>Time spent within 5 m</td>
<td>47.5 ± 79.9</td>
<td>52.8 ± 58.1</td>
<td>0.034 $_{1,22}$</td>
<td>0.856</td>
</tr>
<tr>
<td>Distance of closest approach</td>
<td>7.3 ± 4.4</td>
<td>6.3 ± 3.3</td>
<td>0.423 $_{1,22}$</td>
<td>0.523</td>
</tr>
<tr>
<td>Distance at end of playback</td>
<td>12.1 ± 4.8</td>
<td>22.3 ± 13.7</td>
<td>-1.502*</td>
<td>0.140</td>
</tr>
<tr>
<td>Duration of response</td>
<td>183.3 ± 97.4</td>
<td>170.3 ± 104.1</td>
<td>0.100 $_{1,22}$</td>
<td>0.755</td>
</tr>
</tbody>
</table>
Figure 1. Behavioural responses of free-living male golden whistlers to caged decoy males with control ($n = 13$) and reduced ($n = 12$) throat patches during simulated territorial intrusions. Variables are: time spent within 5 m from the cage containing the decoy bird ($F_{1,21} = 4.84$, $p = 0.039$), total length of response during playback ($U = -2.08$, $n_{control} = 13$, $n_{reduced} = 12$, $p = 0.037$) and final distance of focal male from the cage at the completion of the playback period ($U = -2.27$, $n_{control} = 13$, $n_{reduced} = 12$, $p = 0.022$).
Figure 2. The relationship between the difference between the throat patch sizes of the focal male and caged decoy male, and focal male latency in responding to the apparent threat (with apparent outlier: throat difference: $F_{1,11} = 4.707, p = 0.053$; treatment: $F_{1,11} = 0.965, p = 0.347$; without outlier: throat difference: $F_{1,10} = 4.734, p = 0.055$; treatment: $F_{1,10} = 1.158, p = 0.307$). Throat difference was calculated as focal male throat patch size minus decoy male patch size.
Chapter 7

Static versus dynamic signalling during territory defence by golden whistlers (*Pachycephala pectoralis*)

Unpublished manuscript

Abstract

Avian display ornaments vary widely in their sensitivity to changes in signaller condition. Plumage ornaments are typically relatively static, undergoing little change throughout the year. Other traits, such as singing behaviour, are more dynamic, changing regularly with signaller condition or motivation. The relative importance of these traits in signalling individual quality, especially male dominance, is poorly understood. Male golden whistlers (*Pachycephala pectoralis*) display putatively static and dynamic signals during territorial disputes, including a white throat patch (static signal) and high singing rates (dynamic signal). I conducted field-based simulated territory intrusions in which both patch size and apparent singing rates of caged males were experimentally varied to evaluate sex-specific differences in prioritisation of static and dynamic traits. Males paid attention to both categories of signals when estimating rival threat. They responded for longer towards males with large throat patches and spent more time close to the intruder in high song rate trials. Throat patch size may reflect the rival’s long-term viability and competitive abilities, while singing rates may fluctuate with willingness to escalate the contest. In contrast, females only focussed on dynamic signals, displaying more aggression during high song rate trials. Females participate in joint territorial defence, but disputes between neighbours are sex-specific. Knowledge of an intruding male’s competitive abilities may therefore be unimportant. However, females may discern variation in male singing rates if they reflect the motivation of both members of an intruding pair to engage in a contest. Sex-specific differences in responses therefore reflect context-dependent variation in the information content of static and dynamic traits.
Introduction

Birds display a wide range of conspicuous sexual displays that honestly convey information about the signaller’s phenotypic or genetic quality (Andersson 1994). A ubiquitous feature of display ornaments is the high variability in their capability to reflect the signaller’s current condition. Some signals, such as elaborate plumage traits, are relatively static and a long lag period exists between a change in signaller condition and a change in trait expression (e.g. Fitze and Richner 2002). On the other hand, dynamic ornaments, such as singing rates, are highly plastic and much more responsive to short-term variation in signaller condition or motivation (e.g. Birkhead et al. 1998).

The relative importance of static versus dynamic ornaments in signalling male quality is debated. Dynamic traits may be more reliable as indicators of male quality, because such signals respond relatively rapidly to continual variation in male condition and therefore may be a better estimate of current quality (e.g. Folstad and Karter 1992; Mougeot et al. 2004). Alternatively, static traits may be more reliable because they are less sensitive to variation in the factors affecting signal expression, such as condition or willingness to engage in antagonistic contests, and therefore less likely to result in cheating by inferior individuals temporarily expressing a high quality ornament (e.g. Møller et al. 1998; Scheuber et al. 2004).

Studies investigating static and dynamic ornaments concurrently have typically focussed on displays used in female mate choice, and these studies have produced conflicting results. Firstly, individual females may pay attention to different ornaments expressed by male conspecifics. For example, during mate choice, some female dark-eyed juncos (Junco hyemalis) weigh a static plumage ornament more heavily than dynamic courtship displays, while other individuals use the opposite weighting (Hill et al. 1999). Thus non-uniform female preference for traits signalling current body condition (courtship display) or male age (plumage ornament) may, in part, drive the evolution of multiple ornaments in this species. Secondly, females may base mate choice on multiple criteria. In the scaled quail (Callipepla squamata) females prefer both large males and those displaying at high rates, suggesting that knowledge of both a short-term indicator of male condition (display
rate) and a more reliable indicator of male viability (body size) are important as selection
criteria for females (Hagelin and Ligon 2001). Finally, females may pay differential
attention to static and dynamic ornaments depending on the relative expression of those
traits. This may be important if females require additional information regarding signaller
quality if initially basing mate choice decisions on traits that are susceptible to cheating
(inferior males can presumably temporarily express high quality dynamic traits). For
example, in barn swallows (Hirundo rustica), both singing rates and tail streamer length are
important determinants of male extra-pair reproductive success (Møller et al. 1998), but
females only use song rates as a choice cue for males that already have long tail streamers.

Although studies investigating static and dynamic trait use during female mate choice are
relatively common, those focussing on variation in male responses to both categories of
traits during antagonistic contests are much rarer (e.g. Hagelin 2002). This is despite high
variation in the plasticity of different antagonistic signals and vastly different capabilities
of ornaments to signal male long-term fighting ability or short-term motivation to continue
or escalate physical contests. Thus our knowledge of the complex interactions between
static and dynamic traits used during competition for resources is limited. Additionally, in
species where both sexes cooperate in joint territorial defence, sex-specific differences may
exist in whether static or dynamic antagonistic traits are emphasised. This may arise if the
value of information broadcast by the signals differs between the sexes (Fawcett and
Johnstone 2003). For example, if territorial disputes are sex-specific, females may pay little
attention to signals broadcasting an intruding male’s fighting abilities, but may focus on
traits reflecting the motivation of the intruding pair to engage in a territorial contest.

The golden whistler (Pachycephala pectoralis) is an Australian passerine in which both
sexes cooperate in the defence of territories. Although partners regularly intrude into
neighbouring territories together, territorial disputes are sex-specific and individuals only
engage in contests with same-sex rivals (Chapter 2; van Dongen and Yocom 2005). Male
golden whistlers possess complex multicomponent displays incorporating both static and
dynamic antagonistic traits. The throat patch is a static signal that can only undergo
significant change once per year, at the time of moult. Male whistlers assess the relative
throat patch size of rival before engaging in a contest, presumably to minimise costly
fighting between highly mismatched individuals (Chapter 6). In contrast, singing rates are highly dynamic and may vary with the motivation of signaler to continue with a territorial dispute. I conducted a field experiment in which I presented caged males with experimentally altered throat patches and song rates to both males and females during simulated territorial intrusions. I expected that, as in previous experiments, females should ignore the trait signalling male competitive abilities (throat patch size), but should instead focus on singing rates which are a more reliable indicator of the motivation and threat posed by intruding pairs. In contrast, males are expected to place equal emphasis on both traits to gain maximum knowledge of the rival’s competitive abilities.

Methods

The study species and site

The golden whistler is a socially monogamous passerine. Males are highly ornamented and display bright yellow breast plumage, a black crown and chin stripe and a white throat patch. Females possess a uniform grey-brown plumage. This study was carried out between November and December 2003 at Toolangi State Forest, Victoria, Australia (37°31’ S, 145°32’E). Individuals were captured and individually marked with a unique combination of three colour bands. Twenty-four territory-holding pairs were monitored daily throughout the breeding season. All nests were located and monitored every two or three days to determine the breeding status of each pair.

Decoy male capture and manipulation

Males used as live decoys were captured within the same forest but outside the study area. When not used in experiments, males were housed in cages measuring 120 x 60 x 60 cm and provided with meal worms ad libitum. I randomly assigned males to one of two groups – ‘reduced throat patch’ males and ‘control’ males. Throat patches sizes were reduced by using black non-toxic ‘Sharpie’ markers (Sanford, USA) to blacken the outer edges of the white throat patch to resemble the surrounding black plumage. Throat patch area was then calculated by measuring the length and width (in mm) of the patch using dial callipers and...
using the following equation: throat patch area = \( \pi \times (\text{width}/2) \times (\text{length}/2) \). I attempted to reduce patch sizes to the minimum limit of natural variation (approximately 460 mm\(^2\)). Control males were subjected to similar treatment but here I applied clear ‘Setasilk’ dye thinner (Pèbèo, France), so that the size of the throat patch remained the same. After my manipulation, the mean throat patch size of the ‘reduced’ group was significantly smaller than that of the control group (control: 635.6 ± 69.9 SD mm\(^2\); reduced: 465.6 ± 78.7 SD mm\(^2\); \( F_{1,10} = 15.65, p = 0.003 \)). The plumage manipulations did not involve an increase in signal size because all white paints and dyes tested did not convincingly match the spectral colouration of the throat patch. However, studies in which signal sizes are manipulated in only one direction are common and have been able to convincingly show that individuals perceive and change their behaviour relative to variation in the trait of interest (Hagelin 2002; Ferns and Hinsley 2004; Senar et al. 2005; Torres and Velando 2005).

**Playback tracks**

Song recordings for the playback tracks were compiled using a Sony TCD-D8 Digital Audio Tape recorder and Sennheiser ME67 unidirectional microphone. I recorded songs from males singing in areas approximately 15 km from the study site to prevent variation in responses due to familiarity with songs. Eight separate 5-minute playback tracks were made consisting of four different tracks per song rate treatment (i.e. high and low song rates: see below). Each track incorporated 4 song-types recorded from a single male in a continuous loop. The low song rate tracks broadcasted songs at 4 songs per minute and the high song rates tracks at 8 songs per minute. I randomised the tracks used in each experiment.

**Experimental setup**

I performed simulated territorial intrusion (STI) experiments to territory-holding males and females. Each trial consisted of introducing a decoy male with either a control or reduced throat patch (the static trait) and song broadcast at a high or low rate (the dynamic trait).
Four different treatment combinations were therefore possible. All STI trials were conducted during the nest building stage for each subject pair, and between 0730 and 1130 AEST (the peak singing period for this species; pers. obs.). A trial commenced with a 5-minute pre-playback period during which all vocalisations made by the focal individual were recorded. A wire cage (18 x 18 x 36 cm) containing the live decoy male was then placed on a stand (height: 1.2 m) within 55 metres of the focal individual (mean distance from focal individual: 21.8 ± 10.3 SD m). Each treatment was chosen randomly for each simulated intrusion trial. The playback track was broadcast from a Sony CDX-L460X portable car stereo with Realistic 30W speakers placed adjacent to the cage. Songs were broadcast at a volume approximating the natural intensity of golden whistler song (50 dB at 10 m, Lutron SL-4001 sound level meter). The decoy male never sang during the trials. During the 5-minute playback period, two observers were present, but hidden from view. I described the individuals’ behaviour which was then transcribed by the second individual. I recorded the following response attributes: a) latency (s; the time elapsed before the focal individual displayed an obvious response to the STI, such as an approach to the cage); b) the number of songs sung; c) the distance of closest approach (m); d) final distance (m) and e) total duration of the response (s).

I estimated the focal bird’s distance from the cage during the trial using a three-dimensional zoning system. Nine different zones were identified (in a 3 x 3 design) from zone 1 (within 5 m from the cage, both horizontally and vertically) to zone 9 (further than 20 m from the cage horizontally and higher than 15 m above the cage; Fig 1.). Changes in the location of individuals within the zones throughout the trial were dictated onto a cassette recorder. Individual distance from cage was later estimated by calculating the length of the vector spanning the top of the cage to the middle of each zone (i.e. distance from cage = \(\sqrt{d^2 + h^2}\), where \(d\) = the zone’s horizontal distance from cage and \(h\) = the zone’s height above cage). The mean horizontal distance of birds in zones 3, 6 and 9 was estimated to be 30 m, while the mean height above the cage in zones 7, 8 and 9 was estimated to be 20 m. I then estimated average distance of the individual from the cage throughout the trial by calculating the proportion of time spent within each zone i.e. average distance = (proportion of time in zone 1)*(mean distance of zone 1 from cage [3.5
m]) +…+ (proportion of time in zone 9)*(mean distance of zone 9 from cage [36.1 m]). A 5-minute post-playback period followed the playback period in which all male vocalisations were recorded.

Statistical analysis

Due to the large number of trials conducted (n = 96), individuals were inevitably subjected to multiple trials throughout the sampling period (mean number of trials per individual: 2.6 ± 0.7 SD trials; range: 1 – 3). I attempted to conduct trials at least 3 days apart for each individual but since this species is single-brooded and I conducted all trials during the nest building stage, the window of opportunity was often small. Therefore I conducted trials on some individuals on subsequent days (mean number of days elapsed between each trail for an individual: 7.5 ± 9.8 SD days; range: 1 – 44 days). To allow for the non-independent nature of the data I used Generalised Linear Mixed Models (GLMM) incorporating individual identity as a random factor. This controlled for differences in responses between individuals. In all cases, the response variables followed either a normal or Poisson distribution and the models calculated using normal (with identity link) or Poisson (logarithm link) error variances.

When analysing the song output data, I incorporated singing rates during the pre-playback period as a covariate to control for differences in baseline vocal activities before the trials (Hall 2000). All analyses were performed using Genstat 7.0 (Lawes Agricultural Trust, 2003). Experiment-wide Type I error rates were adjusted using Bonferroni correction when multiple tests were conducted. However to reduce the subsequent increased risk of type II errors, I increased the alpha level to 0.1 for these tests only (Wright 1992; Chandler 1995). Data are presented as means and standard deviations unless otherwise stated.
Results

Focal individuals typically did not respond to the simulated intrusions alone. In 93% of all trials involving female subjects (43/46), the male partner also responded. In addition, female partners responded in 64% of trials targeting male subjects ($n = 60$). Males did not change any aspect of their behaviour in the presence of female partners (all $p > 0.05$). I could not conduct a similar analysis for females as males were present during the majority of trials.

Female responses

Females typically responded to the intrusion trials by slowly and quietly approaching the stimulus, although occasionally their responses were more aggressive (e.g. singing in close proximity to the cage; pers. obs.). Females showed some evidence of habituation to the trials. Females that had been subjected to a previous trial did not approach the cage as closely (closest approach: first trial = $8.2 \pm 5.1$ m, second = $11.3 \pm 6.0$ m, third = $14.6 \pm 7.2$ m, $Wald = 6.58$, d.f. = 2, $p = 0.038$) and tended to sing less (song rate during playback: first trial = $1.0 \pm 1.5$ songs/min, second = $0.8 \pm 1.7$ songs/min, third = $0.2 \pm 0.4$ songs, $Wald = 5.82$, d.f. = 2, $p = 0.054$). I therefore included trial number as a co-variate in all female analyses involving these response measures.

Females showed more interest toward high song rate treatments. When songs were broadcast from the speakers at a high rate, females were faster to respond to the threat (song rate – $Wald = 5.90$, $p = 0.015$; throat patch – $Wald = 0.04$, $p = 0.836$; Fig. 2) and approached closer to the cage (song rate – $Wald = 5.69$, $p = 0.017$; throat patch – $Wald = 2.04$, $p = 0.154$; Fig. 3), while their average distance from the cage was also closer (song rate – $Wald = 11.11$, $p < 0.001$; throat patch – $Wald = 1.90$, $p = 0.168$; Fig. 3). The duration of their aggressive response was longer during high song rate trials (song rate – $Wald = 11.98$, $p < 0.001$; throat patch – $Wald = 0.72$, $p = 0.395$; Fig. 2), after which they ended closer to the cage at the trial’s completion (final distance: high song rate = $17.2 \pm 13.4$ m,
low song rate = 28.4 ± 14.6 m; song rate – Wald = 5.99, p = 0.014; throat patch – Wald = 0.83, p = 0.361). These relationships remained significant after Bonferroni corrections were made (Bonferroni-adjusted critical value is p = 0.016).

Females tended to show no difference in their responses to variation in the throat patch size of the caged male. However, they tended to sing more during trials in which the introduced males had a reduced throat patch (pre-playback song rate – Wald = 6.02, p = 0.014, song rate – Wald = 2.68, p = 0.102; throat patch – Wald = 5.44, p = 0.020; Fig. 4).

Male responses

Males did not appear to habituate to the playback trials. Furthermore, there was no difference in behaviour between males that were used for similar simulated territorial intrusion experiments in the previous year and new males (all p > 0.1). Both singing rate and throat patch size had significant effects on the behaviour of the focal males. Males tended to respond more slowly to trials in which caged males had large throat patches. However, an interaction existed between the two treatments such that the effect of throat patch size only applied for high song rate trials (song rate – Wald = 1.09, p = 0.296; throat patch – Wald = 4.59, p = 0.032; song rate*throat patch interaction – Wald = 3.37, p = 0.06; Fig. 5). The closest distance that males approached the cage during the trial was shorter during high song rate treatments (closest approach: high song rate = 10.6 ± 5.5 m, low song rate = 16.7 ± 9.0 m; song rate – Wald = 8.76, p = 0.003; throat patch – Wald = 0.08, p = 0.779). The average distance of focal males throughout the entire trial was also closer during the high song rate treatments (song rate – Wald = 19.35, p < 0.001; throat patch – Wald = 0.22, p = 0.637; Fig. 5). Responses of males tended to be longest during trials using males with large throat patches (song rate – Wald = 1.55, p = 0.214; throat patch – Wald = 3.79, p = 0.05; Fig. 6) and males sang more in response to males possessing reduced throat patches (pre-playback song rate – Wald = 17.82, p < 0.001, song rate – Wald = 2.02, p = 0.156; throat patch – Wald = 5.84, p = 0.016; Fig. 7). Males ended closer to the cage at the completion of high song rate trials (final distance: high song rate = 20.2 ± 14.3 m, low song
rate = 35.7 ± 17.1 m; song rate – Wald = 12.96, p < 0.001; throat patch – Wald = 0.82, p = 0.365). All relationships, except trial duration remained significant after Bonferroni adjustments were made (Bonferroni-adjusted critical value is p = 0.016).

Discussion

Manipulations of throat patch size and singing rates of male golden whistlers during simulated territorial intrusions prompted different reactions from male and female subjects. Female golden whistlers reacted more strongly to high song rates and apparently paid little attention to variation in the size of the throat patch of the introduced male. By contrast, resident males showed clear-cut responses to manipulations of both the throat patch and singing rate of the introduced male.

Throat patches and singing behaviour both function as antagonistic signals during territorial disputes, but these two signals may differ in how reliably they indicate male viability. Variation in plumage traits is relatively small throughout the year and significant changes in plumage expression typically occur only once a year at the time of moult (Payne 1972). Therefore plumage quality may reflect a ‘historical’ state of signaller condition at or before the time of moult. For example, in house sparrows (Passer domesticus), individuals expressing high levels of testosterone prior to the moulting period typically develop high quality status signals (Evans et al. 2000; Gonzalez et al. 2001). Yet, although high testosterone levels are required for enhanced signals, they also increase the signaller’s competitive abilities (Wingfield et al. 1987; De Ridder et al. 2000; Duckworth et al. 2004). Therefore, static plumage traits are typically reliable indicators of long-term male quality and fighting abilities (reviewed by Whiting et al. 2003), although the exact role of testosterone during signal development is still debated (Roberts et al. 2004). In contrast, singing rates readily fluctuate with current motivation during a territorial dispute (Naguib 2005), but may be less reliable signals of long-term viability if inferior individuals are able to temporarily express a high-quality signal (Møller et al. 1998).
The relative importance of static (throat patch) and dynamic (singing rate) signals as indicators of male quality during territorial disputes is still poorly understood. When individuals display multiple ornaments, conspecifics are expected to emphasise those signals that are most informative about aspects of signaler quality relevant to the receiver (Fawcett and Johnstone 2003). This can lead to sex-specific differences in ornament prioritisation if the value of information extracted from signals differs between the sexes. For example, in the highly ornate Gambel’s quail (*Callipepla gambelii*), testosterone-dependent display rates are an important signal in both male competition and female choice because they are thought to accurately reflect male quality or motivation (Hagelin and Ligon 2001; Hagelin 2002). In contrast, head plume size is a poor indicator of male quality and is therefore not female-selected. Yet, this trait reliably signals male dominance and plays a key role in determining the outcomes of male contests (Hagelin 2002).

Female golden whistlers displayed increased interest in intruders singing at high rates, a pattern that may arise for several reasons. First, females may assess the intruders as potential extra-pair mates. During mate assessment, female birds may emphasise static traits (e.g. Hasselquist *et al.* 1996), dynamic traits (e.g. Mennill *et al.* 2002) or both (e.g. Møller *et al.* 1998). Female golden whistlers appeared to focus on a dynamic signal, perhaps because it more accurately reflects the current condition of the intruder. Additionally, females may remember outcomes of contests between neighbouring males to make future extra-pair mate choice decisions (Mennill *et al.* 2002). However, paternity analyses reveal that males singing at high rates throughout the season attract fewer, rather than more, extra-pair fertilisations (Chapter 5). Females are therefore unlikely to show increased interest during high song rate trials for mate choice purposes.

An alternative explanation for female approaches is that they are aggressive and females react more strongly to intruders singing at high rates for territory defence purposes. In many species, both sexes cooperate in defending their territory from intruders. Cooperative defence of territories may arise if joint defence is more effective at deterring intruders or if each member of the pair defends the territory against same-sex rivals (Langmore 1998). During these joint contests, female whistlers only actively chase other females and never engage in aggressive contests with males (Chapter 2, van Dongen and Yocom 2005). This
suggests that the antagonistic ornamentation of male intruders should be of little interest to females. Nevertheless, pairs often intrude onto a neighbouring territory together. Therefore, if a male intrudes onto a neighbouring territory, there is a strong likelihood that his partner will also be present. If territorial intrusions involve coordinated aggression by the members of a pair, and the willingness of both individuals to escalate encounters is correlated, females could use the singing rates of intruding males to predict the motivation of his partner to engage in a territorial contest.

Female golden whistlers tended not to display differential responses to males with different patch sizes. Static antagonistic signals typically reflect the signaller’s competitive abilities and may be assessed by opponents in decisions on whether to engage in costly physical contests (Bradbury and Vehrencamp 1998; Senar 1999). Since females only engage in contests with same-sex rivals, the fighting abilities of males may be irrelevant and only traits that signal pair motivation may be emphasised. Females only changed their singing behaviour in response to variation in intruder throat patch size, singing more in response to caged males with small throat patches. A similar pattern in singing behaviour was observed for males. During joint territory defence against intruding neighbouring pairs, females increase duetting rates with males (unpublished data). Thus, increased singing rates of females may be a secondary response to the higher singing rates of their partners, instead of a direct reaction to the manipulated throat patch.

In contrast to females, both high singing rates and large throat patches induced aggressive responses from males. Surprisingly, ornament assessment did not occur in a linear additive manner. Thus, focal males were not most aggressive towards introduced males possessing both a large throat patch and high singing rates, suggesting males pay attention to each trait separately and for different reasons. By assessing both traits, males may gain information on both the intruder’s long-term competitive abilities and current motivation. This supports the multiple message mechanism for multiple ornamentation proposed by Møller and Pomiankowski (1993), stating that individuals display multiple signals to advertise different aspects of their quality to conspecifics. Variation in the throat patch resulted in differences in response latency and duration, while variation in singing rates was related to response intensity, or how closely the resident male approached the intruder.
Before engaging in a contest, resident males assess the relative throat patch size of the intruder, perhaps to prevent costly fighting between highly mismatched individuals (Chapter 6). Here I have shown that males also assess singing rates during this period: individuals with large patches and high singing rates may be more intimidating to resident males, resulting in a slower response time. The time elapsed before residents gave up attempting to expel an intruder was also dependent on intruder throat patch size, suggesting that patch size is a better indicator of the overall threat of the male. However, the intensity of the resident’s response was unrelated to patch size but varied directly with intruder singing rates, presumably because they reliably reflect intruder willingness to continue the contest.

In a previous study (conducted in 2002, Chapter 6) only throat patches of caged males were manipulated and the results of that experiment can be directly compared to those I obtained in the current study. In both years, the duration of the resident male’s response and response latency were dependent on intruder throat patch size, but only during high song rate trials. However, there are also several differences between the findings of the two experiments. Firstly, the position of the focal individual relative to the cage was much closer during the 2002 trials (i.e. distance of closest approach and final distance). One possible explanation for this difference is that focal males became habituated to the caged male and this reduced the intensity of their response. This seems unlikely because males that were used in both years did not differ in behaviour to males that were used for the first time in 2003. A more straightforward explanation may be that this incongruence simply reflects differences between the experiments in sampling protocols. For example, in 2002, only the focal male’s horizontal distance from the cage was estimated, whereas in 2003, height above the cage was incorporated into distance measures, which were dramatically increased if males were high up in the forest canopy. Yet, recalculating male distances for the 2003 experiments without incorporating vertical height above cage revealed that these differences persist (closest approach: 2002 - 3.9 ± 4.5 m, 2003 – 11.6 ± 9.1 m, F$_{1,74}$ = 16.00, p < 0.001; final distance: 2002 - 10.8 ± 11.0 m, 26.5 ± 18.3 m; F$_{1,74}$ = 8.71, p = 0.004). The most likely explanation is the large difference between years in how closely I placed the cage to the focal male at the commencement of the trial. In 2002, I commenced
trials when focal individuals were 12.4 ± 3.9 m from the cage (data for males only), compared to 23.4 ± 10.0 m in 2003 ($F_{1,74} = 24.26, p < 0.001$).

Resident males also spent more time within five metres from the cage and were closer to the cage at the trial’s termination during large throat patch trials in 2002. There were no such differences in 2003, when only singing rates predicted resident position. Again, this may be due to differences in experimental conditions. In 2002, the percentage difference in mean throat patch sizes between ‘control’ and ‘reduced’ groups (i.e. $[\text{mean control throat size} – \text{mean reduced throat size}] / \text{mean control throat size}$) was 40%, compared to 27% in 2003. Perhaps only relatively large differences in throat patch size are required to detect variation in male ‘intensity’ responses, reinforcing the idea that intruder singing rates are the most important signal predicting male response intensity. Finally, I report here that males sang more in response to small throat patch trials, perhaps since these individuals pose less of a threat and can normally be expelled from the territory by singing alone. No such difference was detected during 2002. The discrepancy appears not to be due to differences in statistical power between years (power: 2002 = 0.145, 2003 = 0.155). Additionally, it is unlikely that the effect detected in 2003 was biologically non-significant, as both sexes sang more in response to small throat patch trials during this year. Finally, no differences in sampling protocols between years provide a convincing explanation for the difference. It is thus difficult to account for this anomaly.

In one of the few studies concurrently investigating the relative importance of static and dynamic traits in male competition, Hagelin (2002) reported that both a plastic cue reflecting male motivation (an erectable head crest) and a static trait (body size) in Gambel’s quail ($Callipepla gambelii$) were important predictors of outcomes of male-male contests. Large males with enlarged crests were more likely to dominate their competitors. Competing male golden whistlers also appear to pay attention to both static and dynamic traits. Moreover, my study demonstrates that subtle variation may occur in male response effort and intensity to different combinations of manipulated traits.

What prevents subordinate golden whistlers with inferior competitive abilities from cheating in the expression of these signals? One possibility is that cheating is regulated via
the high costs of increased aggression directed towards individuals displaying large status signals, a common socially-induced mechanism reinforcing signal honesty (Metz and Weatherhead 1992; Senar 1999). In many species, including the golden whistler, males are more likely to engage in a contest with rivals expressing similar-sized status signals (Chapter 6; Balph et al. 1979; Möller 1987; Pryke and Andersson 2003). Using relative status signal size to make an initial assessment of rival threat may prevent costly fighting between highly mismatched individuals. Therefore, subordinates expressing a dishonestly large status signal will attract more frequent attacks from dominant individuals with superior competitive abilities (Maynard Smith and Harper 1988). These aggressive attacks are associated with many costs, including increased risk of injury, increased energy expenditure and exposure to predators (Whiting et al. 2003), and these costs have been shown to be greater among low quality individuals (e.g. Veiga 1995). Such a system may be particularly important for policing cheating with respect to singing, which probably imposes only minimal physiological costs on the signaller (Oberweger and Goller 2001; Franz and Goller 2003; Ward et al. 2003). Although social control presumably plays an important role in regulating throat patch size, high physiological costs may also be incurred in the production of this plumage signal. The expression of status signals is often under the control of testosterone, which not only increases male aggression but also reduces signaller condition via strong immunosuppressive effects (Folstad and Karter 1992, but see Roberts et al. 2004 for studies reporting conflicting results). Nevertheless, such physiological costs may be particularly important if the benefits gained by signal deception outweigh the costs associated with occasional attacks from conspecifics (Slotow et al. 1993).

In conclusion, I have shown experimentally that in golden whistlers, both throat patch size and singing rates are assessed by conspecifics during elevated territorial disputes. Females display increased aggression towards intruders singing at high rates, but ignore variation in throat patch size. In contrast, both high song rate and large throat patches elicited strong responses among males. Thus striking sex-specific differences exist in how plastic and dynamic traits are weighed during territorial disputes, suggesting that each sex emphasises different aspects of male long- and short-quality. Additional research on sex-specific differences in the emphasis of long- and short-term indicators of male quality is required to further our knowledge on the selection pressures imposed by each sex on the two ornament
types. Furthermore, this will reveal novel insights into the evolution of multicomponent sexual displays and the complex interactions that occur in the expression of those ornaments.
**Figure 1.** The three-dimensional zoning system used during simulated territorial intrusions to describe the position of focal golden whistlers relative to a cage containing the decoy male. For example, individuals were in zone 4 when within 5 m of the cage horizontally and between 5 and 15 m above the cage. Changes in the location of individuals within the zones were dictated onto a cassette recorder throughout the trial. The focal individual’s average distance from the cage during the trial was later estimated via the proportion of time spent within each zone (see Methods).
Figure 2. Temporal responses of female golden whistlers to caged males during simulated territorial intrusions. Caged males possessed either a large (control) or reduced throat patch, and song was broadcast from speakers at either high or low rates. Latency describes the time elapsed before the female responded to the trial (song rate – Wald = 5.90, p = 0.015; throat patch – Wald = 0.04, p = 0.836). Duration of response was the temporal difference between the first and last responses (song rate – Wald = 11.98, p < 0.001; throat patch – Wald = 0.72, p = 0.395). Horizontal bars and values indicate the fitted values derived from the GLMM model. Error bars represent standard error.
Figure 3. Behavioural responses of female golden whistlers to caged males during simulated territorial intrusions. Caged males possessed either a large (control) or reduced throat patch, and song was broadcast from speakers at either high or low rates. Average distance from the cage was calculated via continual estimations of distance throughout the trials (song rate – Wald = 11.11, p < 0.001; throat patch – Wald = 1.90, p = 0.168). Closest approach was the closest distance to the cage attained by the female during the trials (song rate – Wald = 5.69, p = 0.017; throat patch – Wald = 2.04, p = 0.154; trial – Wald = 6.58, p = 0.038). Horizontal bars and values indicate the fitted values derived from the GLMM model. Error bars represent standard error.
Figure 4. Female golden whistler singing rates during the playback period of simulated territorial intrusions (pre-playback song rate – Wald = 6.02, p = 0.014, song rate – Wald = 2.68, p = 0.102; throat patch – Wald = 5.44, p = 0.020). Caged males possessed either a large (control) or reduced throat patch, and song was broadcast from speakers at either high or low rates. Horizontal bars and values indicate the fitted values derived from the GLMM model. Error bars represent standard error.
Figure 5. Responses of male golden whistlers to caged males during simulated territorial intrusions. Caged males possessed either a large (control) or reduced throat patch, and song was broadcast from speakers at either high or low rates. Latency describes the time elapsed before the male responded to the trial (song rate – Wald = 1.09, p = 0.296; throat patch – Wald = 4.59, p = 0.032; song rate*throat patch interaction – Wald = 3.37, p = 0.06). Average distance from the cage was calculated via continual estimations of distance throughout the trials (song rate – Wald = 19.35, p < 0.001; throat patch – Wald = 0.22, p = 0.637). Horizontal bars and values indicate the fitted values derived from the GLMM model. Error bars represent standard error.
Figure 6. The duration of male golden whistler responses during simulated territorial intrusions. Caged males possessed either a large (control) or reduced throat patch, and song was broadcast from speakers at either high or low rates (song rate – Wald = 1.55, p=0.214; throat patch – Wald = 3.79, p = 0.05). Horizontal bars and values indicate the fitted values derived from the GLMM model. Error bars represent standard error. Note that these differences became non-significant when Bonferroni adjustments were made (Bonferroni adjusted critical value is p = 0.016).
Figure 7. Male golden whistler singing rates during the playback period of simulated territorial intrusions (pre-playback song rate – Wald = 17.82, p < 0.001, song rate – Wald = 2.02, p = 0.156; throat patch – Wald = 5.84, p = 0.016). Caged males possessed either a large (control) or reduced throat patch, and song was broadcast from speakers at either high or low rates. Horizontal bars and values indicate the fitted values derived from the GLMM model. Error bars represent standard error.
Chapter 8

General discussion

The findings of this thesis contribute to our understanding of the breeding biology of the golden whistler, the function of visual and acoustic signals in this species, and the function of multicomponent displays in birds more generally. In the following sections, I discuss my findings in light of our current knowledge of multiple ornamentation in birds, and offer suggestions for future research.

Golden whistler ornaments convey ‘multiple messages’

In golden whistlers, multicomponent signalling appears to have evolved primarily via multiple signalling mechanisms and not unreliable or backup signalling. Most other studies investigating multiple ornaments in birds have similarly found evidence for a multiple signalling function (Candolin 2003). Thus, there is growing evidence that this may be the most widespread explanation for multicomponent displays.

Song rate and repertoire, throat patch size and breast colour appear to be the most important components of whistler displays, while the nape band, chin stripe and brightness of the throat patch seem not to be under strong selection. Since there was no inter-correlation between the expression of the four principal display ornaments, conspecifics presumably assess each independently to obtain information on different aspects of male quality. Male breast plumage and song repertoire size seem to function as short- and long-range cues, respectively, during female mate choice. The throat patch, singing rates and interactive vocal behaviours encompass both short- and long-range displays, signalling male dominance during male contests.

The size of the yellow nape band and black chin stripe were not associated with any aspect of male quality and thus could be unreliable, i.e. not currently reflecting any aspect of male
quality and possibly being vestiges of past selection pressures (Candolin 2003). However, the area of olive feathers extending into the yellow nape is highly variable and I did not quantify colour variation in this ornament. Thus if equal concentrations of carotenoids are deposited into the breast and nape plumage, then the colour attributes of these ornaments will be highly inter-correlated and females could assess carotenoid-based plumage variation using both signals. The black chin stripe may not signal an aspect of quality. Instead it may increase the contrast around the white throat patch, facilitating in the assessment of this ornament (e.g. Mennill et al. 2003).

**Acoustic and visual ornaments are important to both males and females**

Several golden whistler ornaments appear to convey information to males, including the static throat patch, dynamic singing rates and interactive vocal behaviours. Throat patch size may reflect the long-term viability and competitive abilities of males and be used to assess the relative threat of rivals during close-range male contests. This was confirmed via experiments revealing that only males responded to variation in throat patch size. By contrast, males appear to advertise their motivation during such contests by varying their singing rates and singing interactively with the rival. Intrusion experiments revealed that variation in responses to different antagonistic signals can be subtle and would have been missed if only winners and losers of disputes over resources were quantified (e.g. Hagelin 2002). Singing rates appeared furthermore to be used in long-distance communication and were highest during the breeding period, but did not appear to advertise male quality to potential extra-pair mates. Instead, low quality males may invest more into singing to guard against the risk of cuckoldry. High singing rates subsequent to the fertile period could alert neighbouring females to a male’s presence (after which other cues are used for mate choice) or play a role in territory defence if more resources are required for offspring provisioning during this time. Experimental work will be required to clarify the role of song in paternity guarding and extra-pair mate choice.

Females appeared to assess the song repertoire size and breast colour of both social and potential extra-pair mates. This contrasts the pattern in some other species, where different cues are used in social versus extra-pair mate choice decisions (e.g. great reed warblers,
Acrocephalus arundinaceus; Catchpole 1986; Bensch and Hasselquist 1992) and suggests that female whistlers may gain both direct and indirect benefits from mating with males with high quality ornaments. The lack of any interaction between the two ornaments in predicting reproductive success implies that females assess the two traits separately to gain information about different aspects of male quality. Differences in the effectiveness of the two signals over large distances may also mean that females select males via a hierarchical process, listening to song repertoires while still in their territory and then visiting a subset of preferred males to assessing breast colour in close proximity. Breast plumage quality and song repertoire size may be preferred signals for females because they are static traits that reflect male viability more reliably than other signals such as singing rates (e.g. Møller et al. 1998).

Future directions

What mechanism underlies signal elaboration?

Elevated testosterone levels are generally required for the enhanced expression of both antagonistic and epigamic displays (e.g. Duckworth et al. 2004; Mougeot et al. 2004). Yet if circulating levels of testosterone control the expression of more than one ornament, then we might expect their expression to be correlated in a manner consistent with the ‘backup message’ hypothesis. Given that the multi-component displays of golden whistlers are not obviously inter-correlated, circulating levels of testosterone either affect the expression of different ornaments in complex and uncorrelated ways, or other mechanisms play an important role in ornament expression. Quantifying links between testosterone profiles and ornament expression would be a useful first step in identifying the proximate factors controlling the expression of multiple signals in golden whistlers. Understanding temporal variation in testosterone levels may also be helpful for an understanding of the high variation in singing rates throughout the breeding season and to reveal the sensitive period for plumage signal size determination (i.e. prior to or during the moulting period).
Testosterone levels could also be experimentally manipulated prior to moulting, in aviary-based experiments. Golden whistlers can readily be kept in captivity (Chapter 6, 7). Trials could compare the expression of male display traits in a testosterone-elevated group in which males are implanted with silastic testosterone-filled implants, a testosterone-reduced group in which males are gonadectomised (gonads are removed via surgery), and a sham-treated group. Since testosterone is thought to influence male competitive abilities (e.g. Duckworth et al. 2004), the testosterone manipulation experiments could be coupled with dominance trials. If the size of the throat patch is manipulated within the treatment groups (e.g. reduced in some of the testosterone-elevated groups and enhanced in some of the testosterone-reduced group), information can be gathered on the relative importance of status signalling and competitive fighting abilities during male contests.

**Why do males singing at high rates sire fewer extra-pair young?**

My finding that males singing at the highest rates during the female fertile period sired fewer extra-pair young contradicts the general expectation that high singing rates are correlated with male quality and are used by females during extra-pair mate choice (Møller 1991b). Furthermore, it is unclear why males continue to sing at high rates subsequent to the fertile period, when singing rates are unrelated to reproductive success. The exact role of high singing rates during the breeding period thus remains unclear and could be tested via playbacks that experimentally enhance the apparent song rates of free-living males during different breeding stages. A song-reduced treatment group could be created by temporally muting some individuals (Westcott 1992; Pytte and Suthers 1999). If song is used for brood guarding and not to advertise quality to potential extra-pair mates, males with enhanced singing rates during the female fertile period should have increased within-pair reproductive success but not extra-pair reproductive success. Song rate enhancement during the incubation stage should have no effect on a male’s ability to gain extra-pair fertilisations.
How do multiple signals in males develop?

Golden whistlers exhibit delayed plumage maturation, and do not moult into full adult plumage until their third year (Chapter 2; van Dongen and Yocom 2005). Birds may delay the acquisition of adult plumage for several reasons, including a) advertisement of subordinate status, thus reducing aggression from adults (Lyon and Montgomerie 1986), b) the dishonest mimicking of females or juveniles to obtain territories or sneak copulations with females (Rohwer et al. 1980) or c) an inability of younger males to bear the high costs of plumage production (Hill 1996). As a consequence, younger males exhibit a more restricted set of display components than adults and the relative importance of visual and acoustic displays may be age-dependent. My research did not focus on sub-adults, but I nevertheless found that these males engaged in close-range encounters with adults (pers. obs.), successfully defended territories and sired 43% of nestlings within their nests ($n = 4$ nests; unpublished data). Since sub-adults are capable of defending a territory and breeding before developing elaborate plumage displays, it is interesting to ask whether the signalling strategies of these individuals are different from adult males, and whether in the absence of plumage signals, conspecifics emphasise singing rates and song repertoires when assessing these males during male competition and mate choice. More can be learnt about the signalling strategies of sub-adult males by quantifying their singing rates, song repertoires, territory size and paternity. Additionally, testosterone manipulation experiments conducted on both adults and sub-adults prior to their moult into definitive adult plumage may well be illuminating in identifying age-related differences in the physiological costs of maintaining high testosterone levels and provide insights into proximate mechanisms controlling delayed plumage maturation in this species.


