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Integrative avian taxonomy and the role of competition in the diversification of passerine birds

by

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B.Sc.(Hons), Trinity College Dublin, 2010

A thesis submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

School of Natural Sciences
(Department of Zoology)

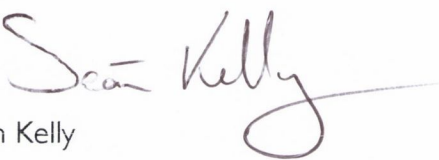
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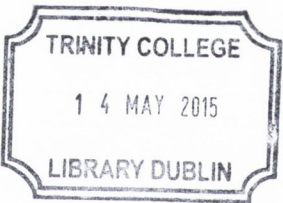
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Summary

It is well recognised that the avifauna of Asia are in major need of taxonomic reassessment. The Sulawesi region of Indonesia, renowned for its high levels of avian endemism, has been relatively overlooked by ornithological researchers. Combining genetic, phylogenetic and morphological analyses, I reassessed the taxonomic relationships of avian populations from peninsular South-east Sulawesi and the nearby Wakatobi Islands. These integrative taxonomic comparisons describe the flowerpeckers (Dicaeidae) of the Wakatobi Islands as a genetically and phenotypically distinct species. I therefore advocate the reclassification of these populations as *Dicaeum kuehni*, the Wakatobi flowerpecker. Furthermore, my results describe the olive-backed sunbird populations present on the Wakatobi (*Cinnyris jugularis infrenatus*) as an incipient species, diverging from its relatives on mainland Sulawesi. Together with the lack of study in the region, my results suggest that species richness and endemism in Sulawesi are underestimated and underscore the need for further integrative taxonomic research.

As the Wakatobi archipelago hosts divergent avian lineages, it offers unique opportunities to study the process of diversification in island birds. To gain further insight into how competitive interactions among organisms drive diversification, I investigated patterns of inter-island phenotypic divergence. I studied the behaviour, ecology and morphology of two ecologically similar white-eye species (Zosteropidae) in sympatry and allopatry. My results suggest that competition with the larger 'Wangi-wangi' white-eye (*Zosterops* sp.) has driven the adaptive divergence of lemon-bellied white-eye (*Z. chloris*) bill size and feeding ecology (i.e. ecological character displacement). Finally, I investigated whether inter-island divergence in *Z. chloris* and *C. jugularis* body size correlated with competition for resources. My results suggest that competition for resources is a major predictor of body size variation and that inter- and intra-specific competition may exert different selection pressures depending on the species' ecology. These results further highlight the powerful role competitive interactions can play in phenotypic diversification.

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My parents and family have always encouraged my interest in the natural world and not only told me to chase my dreams but provided the support to do so. I can very happily say that I am living my dream and, for that, I will never be able to thank you enough.

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1.1 Integrative taxonomy and species delimitation

Taxonomy, the classification of both extinct and extant organisms based on their evolutionary relationships, is central to evolutionary biology. Since the seminal works of Linnaeus (1770), and Darwin and Wallace (1858), taxonomy has helped us to further understand the evolutionary history of life and the processes that have driven it, including the evolution of humans and our ancestors. The field of taxonomy itself has evolved through time, progressing from traditional methods of morphological comparisons of deceased specimens, to a modern, integrative taxonomy that combines traditional methods with data on the genetics, phylogenetics, behaviour, ecology and distribution of organisms to infer evolutionary relationships (e.g. Padial and De, 2010). The advent of DNA sequencing and contemporary phylogenetic methods have greatly improved our understanding of evolutionary relationships (Bickford et al., 2007). Today, we can more accurately ascertain the relationships within an entire class (e.g. Jetz et al., 2012), between different geographic populations of the same species (e.g. Lohman et al., 2010), or between individuals within a population (e.g. Grant and Grant, 2008). However, as no single method is without its flaws, DNA sequencing has not been the panacea of taxonomy and modern taxonomy is best conducted using multiple lines of evidence (Rubinoff and Holland, 2005).

Species are arguably the most important units in taxonomy, macro-evolutionary research and conservation biology. Therefore, with the development of taxonomy into a multidisciplinary field came a vast array of operational definitions for what constituted a species and how species should be delimited. These range from the widely supported biological species concepts to ecological, phylogenetic and phenetic species concepts (see de Queiroz, 2007). I believe modern taxonomy can rely on the so-called unified or general lineage species concept (de Queiroz, 2007), which has been adapted by ornithological taxonomists to develop quantitative criteria for species delimitation (Tobias et al., 2010). This unified species concept retains and promotes an idea common to other species concepts, i.e. that species are independently evolving lineages, but it differs from other

species concepts in that it makes this the only required attribute of a species (de Queiroz, 2007). Under this concept, all other species properties such as monophyly, reproductive isolation, morphological and ecological distinction are not necessary characteristics but are used as evidence of lineage divergence in a taxonomic investigation. Under the unified species concept, which I use hereafter, the aforementioned properties, as well as many others, are the means of species delimitation. To determine if the extent of divergence among lineages is indicative of a species-level difference, observed divergence levels can be compared to the typical degree of divergence between well recognised, closely related species.

Armed with the toolkit of modern taxonomy, evolutionary biologists have identified a multitude of unique species that were either erroneously classified as members of another taxon or simply went unnoticed due to a lack of detailed sampling or observation previously (i.e. so-called cryptic species; see Bickford et al., 2007). The resolution of taxonomic relationships is essential to the development of a greater understanding of the processes that drive speciation and the speed at which diversification occurs across different taxonomic groups (e.g. fish: Schluter (1995); birds: Melo et al. (2011); and amphibians: Setiadi et al. (2011)). Furthermore, the description of previously cryptic species can have implications for conservation, agriculture and healthcare practices at local and regional scales (Beheregaray and Caccone, 2007; Bickford et al., 2007). The tools of modern taxonomy also allow one to identify incipient species, i.e. those lineages that have diverged from their ancestors but do not warrant recognition as a separate species, and to monitor patterns and processes of divergence through time (e.g. Grant and Grant, 2006).

1.2 The role of competition in adaptive divergence and speciation

Speciation, the complex process by which new species arise, is understood to be driven by a suite of environmental and biological factors, from landscape changes that create barriers to gene flow, to differences in levels of predation that drive divergence (Price, 2008). Competition for niche space, i.e. the physical and biological conditions required to grow, survive and reproduce (*sensu* Hutchinson, 1957), has long been hypothesised as a driver of divergence between and within species. In fact, Darwin (1859) was the first to propose competition as the primary engine of diversification. Today competitive interactions among organisms are understood to play a major role in the generation,

maintenance and loss of biodiversity (Pfennig and Pfennig, 2012b). While competition isn't the only biological process thought to promote diversification, it may be one of the most common as it can, uniquely, be mutually costly to all parties involved (when it is not asymmetric); whereas predation, parasitism, mutualism and other processes can not (see Pfennig and Pfennig, 2012b).

Competition is hypothesised to be most intense between ecologically and morphologically similar species. When such competition occurs, one species may drive the exclusion of the other (Gause, 1934; Hardin, 1960; Pigot and Tobias, 2013). Alternatively, in such a competitive situation, selection favouring those individuals who overlap least in niche space will drive adaptive divergence in one or both populations; thus facilitating co-existence. This competition-driven divergence in resource exploiting traits is known as ecological character displacement (Brown and Wilson, 1956; Grant, 1972) and has strong support from both empirical and theoretical research (see Pfennig and Pfennig, 2012b). The process of ecological character displacement was most famously illustrated by Grant and Grant (2006) in a study of Darwin's Finches in which reduced resource availability led to increased interspecific competition between two *Geospiza* species and the survival of only those divergent phenotypes which overlapped least in resource use and bill morphology.

While competition is understood to be a primary driver of divergence between species, direct and indirect competitive ecological interactions can also drive diversification within species. For example, Winkelmann et al. (2014) demonstrated experimentally that niche competition can promote divergent selection and maintain reproductive isolation via assortative mating and thus explain the divergence of genetically and phenotypically distinct lineages of cichlid fish. Competition can also drive lineage divergence via directional selection. For example, the dominance hypothesis predicts that when intraspecific competition for resources is high, selection will favour traits that confer a competitive advantage in agonistic interactions, thus driving phenotypic divergence to a new adaptive peak. While there are few empirical examples of this, the most convincing come from studies of island birds (e.g. Scott et al., 2003).

While both inter- and intra-specific competition can promote diversification, interspecific competition can also inhibit range expansions and diversification. For example, Price et al. (2014), in a study of Himalayan bird communities, determined that interspecific competition for niche space limited the rate of diversification. Price et al. (2014) concluded that

when species colonised new niche space they limited the successful colonisation of other species in the area until further niche space became available (i.e. with the continued uplift of the Himalayan mountains). McGuire et al. (2014), in a study of the diversification of hummingbirds, came to similar conclusions; they recorded increased diversification rates as the Andes mountain range and other geological features formed, suggesting that competition for niche space had limited diversification prior to this. Furthermore, Gutiérrez et al. (2014), using empirical data on South American opossums and ecological niche models, and Pigot and Tobias (2013), combining phylogenetic, morphological and distributional data on Furnariidae (ovenbirds and woodcreepers), determined that competitive interactions between species, and not environmental barriers, can explain the observed patterns of allopatry. Contemporary research integrating detailed morphological, ecological, genetic, phylogenetic and other sources of data promises to shed continued light on the powerful role of competition in evolution.

1.3 Avian diversity in Sulawesi and the Wakatobi islands

As a result of its complex geological history, the Sulawesi region of Indonesia has a remarkably high level of avian endemism; 28% of all bird species found here occur nowhere else in the world (Coates and Bishop, 1997). A key example of the distinctiveness of Sulawesi's avifauna is the maleo (*Macrocephalon maleo*), a large megapode, the sole member of its genus, that incubates its unattended eggs in geothermally and solar heated sands and soils (Elliot, 1994). In recent decades, numerous new bird species have been described from the region (e.g. Lambert, 1998a; Rasmussen et al., 2000; Indrawan et al., 2008). The majority of these species were identified and described using traditional phenotypic comparisons, without the application of DNA analysis. This lack of integrative study, along with the general dearth of ornithological sampling, suggests that avian species richness and endemism in the region are probably underestimated. Thus, conservation and management practices in the region may be sub-optimal, and our understanding of taxonomic relationships, extinction threats and diversification in the region can be improved.

The Wakatobi Islands, previously known as the Tukang Besi Islands, lie off the south-eastern peninsular arm of Sulawesi (see Figure 1). A 27 km wide stretch of ocean, that reaches depths of 1500-2000 m (Kaye, 1989), separates the Wakatobi archipelago from Buton Island, the nearest landmass representative of mainland Sulawesi (Smith and Silver,

1991). The Wakatobi islands are uplifted coral islands that sit atop a platform of Australian origin and have never been connected to mainland Sulawesi (Milsom et al., 1999). Unfortunately, current data on the age of the islands is lacking, with the best estimates suggesting the islands are between one and five million years old (see Kelly and Marples, 2010).

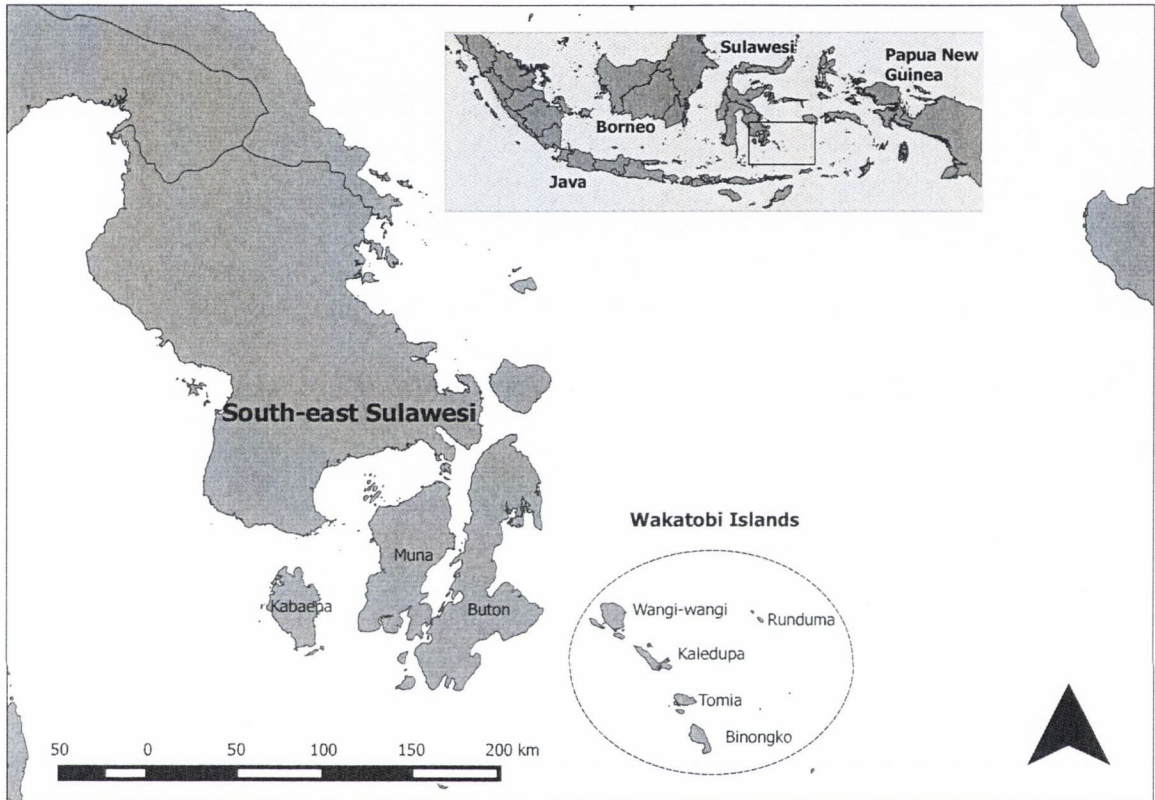


Figure 1.1: Map showing the location of the Wakatobi Islands (circled) relative to peninsular South-east Sulawesi. Inset: the position of Sulawesi relative to other Indonesian landmasses with the enlarged area indicated.

While a previous study had made minor notes on the avifauna of the Wakatobi islands (Meyer and Wigglesworth, 1898), the renowned taxonomist, Ernst J. Hartert, was the first to study the evolutionary relationships of this avifauna. Hartert (1903) originally described four new species, endemic to the Wakatobi, including three new passerine species: a flowerpecker (*Dicaeum kuehni*), a sunbird (*Cinnyris infrenata*) and a white-eye (*Zosterops flavissima*). After several revisions of bird taxonomy and the firm establishment of trinomial nomenclature, all of the aforementioned species were reclassified as endemic subspecies. However, Hartert, the only person to study the taxonomic relationships of the Wakatobi avifauna, used only deceased specimens in his comparisons. Thus, more than 100 years on from Hartert's original descriptions, the birds of the Wakatobi have not been studied using modern taxonomic methods and species delimitation criteria

or live specimens. Furthermore, a new, aberrant white-eye (*Zosterops*) species that is restricted entirely to the island of Wangi-wangi (thus, dubbed the 'Wangi-wangi' white-eye), was discovered during recent surveys of the archipelago (see Kelly and Marples, 2010). This suggests that selection pressures may differ markedly on the Wakatobi relative to mainland Sulawesi and supports Hartert's original claims that the Wakatobi hosts unique, endemic species. Thus, by quantifying the level of genetic and phenotypic divergence between Wakatobi lineages and their closest relatives on mainland Sulawesi, as well as the levels of divergence within Wakatobi lineages, one can reassess the taxonomic relationships of these taxa and test evolutionary theories on the drivers of diversification in island birds.

1.4 Research outline

In this thesis, I draw on a wealth of morphological data and tissue samples¹, as well as detailed behavioural and ecological data, of birds from the Wakatobi archipelago and South-east Sulawesi to reassess the taxonomic status of two Wakatobi lineages and investigate the role of ecological competitive interactions in the divergence of island birds.

In Chapter 2 and Chapter 3, I combine estimates of genetic, phylogenetic and morphological divergence to reassess the taxonomic status of Wakatobi populations of grey-sided flowerpecker (*Dicaeum celebicum kuehni*) and olive-backed sunbird (*Cinnyris jugularis infrenatus*), respectively. The results from Chapter 2 describe the flowerpecker populations on the Wakatobi as a distinct species and I advocate the recognition of these populations as *Dicaeum kuehni*, a species endemic to the Wakatobi Islands. The results from Chapter 3 describe the sunbird populations as an incipient species, diverging from their close relatives on mainland Sulawesi but not warranting recognition as a separate species. Based on the pattern of divergence in both lineages, I hypothesise on the mechanisms that have driven divergence in these populations.

In Chapter 4, I investigate whether competitive interactions between the ecologically similar lemon-bellied white-eye (*Zosterops chloris*) and 'Wangi-wangi' white-eye (*Zosterops sp.*) have driven adaptive divergence in *Z. chloris*' phenotype. These novel results show that, in sympatry with its dominant congener, *Z. chloris* has a shorter bill. The associated behavioural and isotopic data suggest this is an adaptive shift to feeding on smaller invertebrate prey types. This adaptive divergence supports the theory of ecological char-

acter displacement and provides further evidence that interspecific interactions between organisms can drive divergence.

In Chapter 5, I further investigate the role of competition in the generation and maintenance of phenotypic divergence. I compare inter-island body size variation in both lemon-bellied white-eye and olive-backed sunbirds against measures of potential for inter- and intra-specific competition. The results suggest that variation in lemon-bellied white-eye body size is driven by intraspecific competition for food resources and provide support for the dominance hypothesis. By comparison, variation in olive-backed sunbird body size is driven by interspecific competition; specifically, by the presence of the 'Wangi-wangi' white-eye on Wangi-wangi Island. These results provide additional evidence of the powerful role of competition in the evolution of island birds.

¹The majority of morphological data and feather samples used in this research were collected by Dr. Nicola Marples and Dr. David Kelly, with the help of their many volunteers, during various field expeditions between 1999 and 2012. I assisted in the collection of morphological data and samples in 2012. All data were collected under permit and approval from Kementerian Negara Riset dan Teknologi (RISTEK), the Indonesian Institute of Science (LIPI) and Direktorat Jenderal Perlindungan Hutan dan Konservasi Alam (PHKA), the Wakatobi National Park and the Wakatobi Regency.

chapter 2

Molecular and phenotypic data support the recognition of the Wakatobi flowerpecker (*Dicaeum kuehni*) from the unique and understudied Sulawesi region

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Author contributions: Conceived and designed the project: SBAK, DJK, NC, AB, KA and NMM. Performed the field and lab work: SBAK, DJK, NC and NMM. Analysed the data: SBAK and DJK. Contributed reagents/materials/analytical tools: SBAK, DJK, NC and NMM. Wrote the paper: SBAK, DJK, NC, AB, KA and NMM.

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2.1 Abstract

Accurate estimates of species richness are essential to macro-ecological and macro-evolutionary research, as well as to the effective management and conservation of biodiversity. The resolution of taxonomic relationships is therefore of vital importance. While molecular methods have revolutionised taxonomy, contemporary species delimitation requires an integrative, multi-disciplinary approach. Despite boasting a remarkably high level of endemism, the avifauna of the Sulawesi region of Indonesia remains poorly studied. Previous studies of avian diversity in Sulawesi have focussed predominantly on phenotypic characteristics, thus potentially overlooking any genetically distinct lineages. Grey-sided flowerpecker *Dicaeum celebicum* populations from the Wakatobi archipelago were originally described as a separate species from those on nearby mainland Sulawesi. However, for reasons that remain unknown, the Wakatobi populations were reclassified as a subspecies of the mainland form. Combining estimates of genetic divergence with phylogenetic and morphological analyses, we reassessed the status of Wakatobi populations. Our results describe the Wakatobi populations as a separate species to those on mainland Sulawesi; reproductively isolated, genetically and morphologically distinct. We therefore recommend the reclassification of these populations to their original status of *Dicaeum kuehni* and propose the vernacular name 'Wakatobi flowerpecker'. In consideration of our findings and the lack of integrative ornithological research within the Sulawesi region, we believe species richness and avian endemism within the region are underestimated.

2.2 Introduction

Species are the fundamental unit of analysis within ecological, evolutionary and biodiversity research, as well as conservation policy, so accurate estimates of species richness are essential. The presence of erroneously classified species, taxonomic inconsistencies and cryptic diversity are therefore of great significance to these estimates. Accurate species richness data are essential for analysing and understanding patterns of biodiversity (Isaac et al., 2004), as well as for the global designation and conservation of 'biodiversity hotspots' (Myers et al., 2000). Failure to resolve taxonomic relationships and identify unique lineages may result in the ill-informed distribution of conservation resources, the neglect of certain regions and/or species, and potentially the extinction of species before they are even recognised. Advanced taxonomic knowledge is vital to further understand the complex processes that promote lineage diversification, such as the causes of morphological stasis and mechanisms of mate recognition and choice. Furthermore, such studies may uncover previously unknown ecological relationships (Blair et al., 2005) and provide further insight into the factors governing them (Dennis and Hellberg, 2010).

The understanding of taxonomic relationships has improved dramatically in recent decades, primarily due to improvements in genetic sequencing methods and decreases in associated costs (Beheregaray and Caccone, 2007; Bickford et al., 2007). This has allowed the easy delimitation of genetically and phylogenetically distinct lineages. For example, analyses of the mitochondrial 'barcoding' gene COI (cytochrome c oxidase subunit I) have revealed numerous genetically divergent bird lineages (Kerr et al., 2007; Tavares and Baker, 2008; Lohman et al., 2010). However, while genetic analyses provide one of the simplest and most effective methods for resolving taxonomic relationships, they are best combined with traditional phenotypic analyses (Rubinoff and Holland, 2005). Thus, taxonomic reassessments will be most fruitful if based upon an integrative, multi-disciplinary approach, combining genetic and phylogenetic analyses with comparisons of phenotypic traits, such as morphology, ecology, acoustics and other non-visual mating signals (McKay et al., 2010; Melo et al., 2010; Funk et al., 2012).

While a considerable proportion of the world's avifauna is known to be incorrectly classified, the birds of Asia are acknowledged as particularly deserving of major reassessment (BirdLife International, 2013a). As well as demonstrating the need for future research into

the systematics of Asia's avifauna, Lohman et al. (2010) demonstrated the potential for the discovery of currently unrecognised diversity. The avifauna of the geologically complex Sulawesi region of Indonesia remains poorly studied despite boasting a remarkably high level of endemism (16 endemic genera; 28% species endemism) (Coates and Bishop, 1997) and facing significant threats of biodiversity loss (Sodhi et al., 2004). Several new species have been described from the Sulawesi region in the last 20 years or so. However, the focus of study has been primarily in the north of the region, with new species described from the Talaud (Lambert, 1998a,b), Sangihe (Lambert and Rasmussen, 1998; Rasmussen et al., 2000) and Togian islands (Indrawan and Somadikarta, 2004; Indrawan et al., 2008), as well as northern mainland Sulawesi (Rasmussen, 1999). Unfortunately, the majority of such studies in the region relied solely on comparisons of phenotypic traits. In the absence of the integration of genetic and phylogenetic analyses, it is possible that studies of avian diversity in Sulawesi have overlooked a significant number of genetically distinct lineages.

The grey-sided flowerpecker populations from the Wakatobi archipelago, *Dicaeum celebicum kuehni*, were originally described as a separate species (*D. kuehni*) from those on mainland Sulawesi (*D. c. celebicum*) using only phenotypic criteria (Hartert, 1903). For reasons that remain unclear in the literature, the Wakatobi populations were later subsumed as a subspecies of *D. celebicum*. Here, we employ a multi-disciplinary approach, combining genetic, phylogenetic and phenotypic analyses to investigate geographical variation within the grey-sided flowerpecker species and determine whether the Wakatobi populations represent an incorrectly classified, and currently unrecognised, species.

2.3 Methods

2.3.1 Sampling

For this study, we sampled grey-sided flowerpeckers from seven sites across the Wakatobi archipelago, Buton Island and the south-eastern peninsula of Sulawesi (Figure 2.1). The Wakatobi islands are located off the coast of peninsular south-east Sulawesi, approximately 27 km from Buton island, the nearest landmass representative of mainland Sulawesi (Smith and Silver, 1991); thus, from herein, when Sulawesi is referred to, it includes Buton island. In total, 58 grey-sided flowerpeckers (16 female and 36 male) were mist-netted from Buton and South-east Sulawesi (N = 29) and the Wakatobi islands (N = 29) during the months of July, August and September in various years between 1999 and 2012.

When possible, each bird was assessed for age (adult or juvenile) and sex. The following morphometric measurements were taken for each individual (by NMM only): wing length (maximum chord), weight, bill length (to skull), bill depth (at deepest), tail length (standard), tarsus length ('minimum') and total head length (head plus bill) (Redfern and Clark, 2001). Prior to release, a small cluster of contour feathers were plucked from the flank of each bird.

2.3.2 DNA Sequencing

DNA was extracted from feathers using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, California, USA). Extraction followed the manufacturer's protocol except for the final step where bound DNA was eluted twice from the spin column using 150 μ l of Buffer AE instead of 200 μ l. A 672 bp region of the COI gene was amplified using barcoding primers Passer-F1 and Passer-R1 (Lohman et al., 2009). The complete mitochondrial gene ND3 (NADH dehydrogenase subunit 3; 351 bp) was amplified using primers L10755-F and H11151-R (Chesser, 1999) which have proved successful in other flowerpecker studies (Nyári et al., 2009; Fjeldsa et al., 2010). All polymerase chain reaction (PCR) amplifications were performed in 20 μ l reactions consisting of 11.3 μ l double-distilled H₂O, 0.4 μ l 10 mM deoxynucleoside triphosphates (dNTPs), 2 μ l 10x PCR reaction buffer, 1.2 μ l 50 mM MgCl₂, 1 μ l 10 μ M forward primer, 1 μ l 10 μ M reverse primer, 0.1 μ l Taq polymerase and 3 μ l template DNA. All COI reactions were amplified under the following thermal cycler conditions: 4 min at 94°C followed by 35 cycles of 1 min at 94°C, 1.5 min at 58°C and 1.5 min at 72°C, finishing with 5 min at 72°C (adapted from Kerr et al., 2007). Thermal cycling for ND3 ran for 45 cycles instead of 35 to ensure detection of even trace amounts of DNA and the second step of this cycling was lowered from 58°C to 55°C (adapted from Chesser, 1999). PCR products were then cleaned using ExoSAP protocol (Werle et al., 1994), cycle-sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, California, USA), subsequently cleaned with BigDye XTerminator Purification Kit (Applied Biosystems, California, USA) and sequenced in both directions on an Applied Biosystems 3130xl genetic analyzer. All sequences were submitted to GenBank (Benson et al., 2013) under accession numbers JX067909-JX067924.

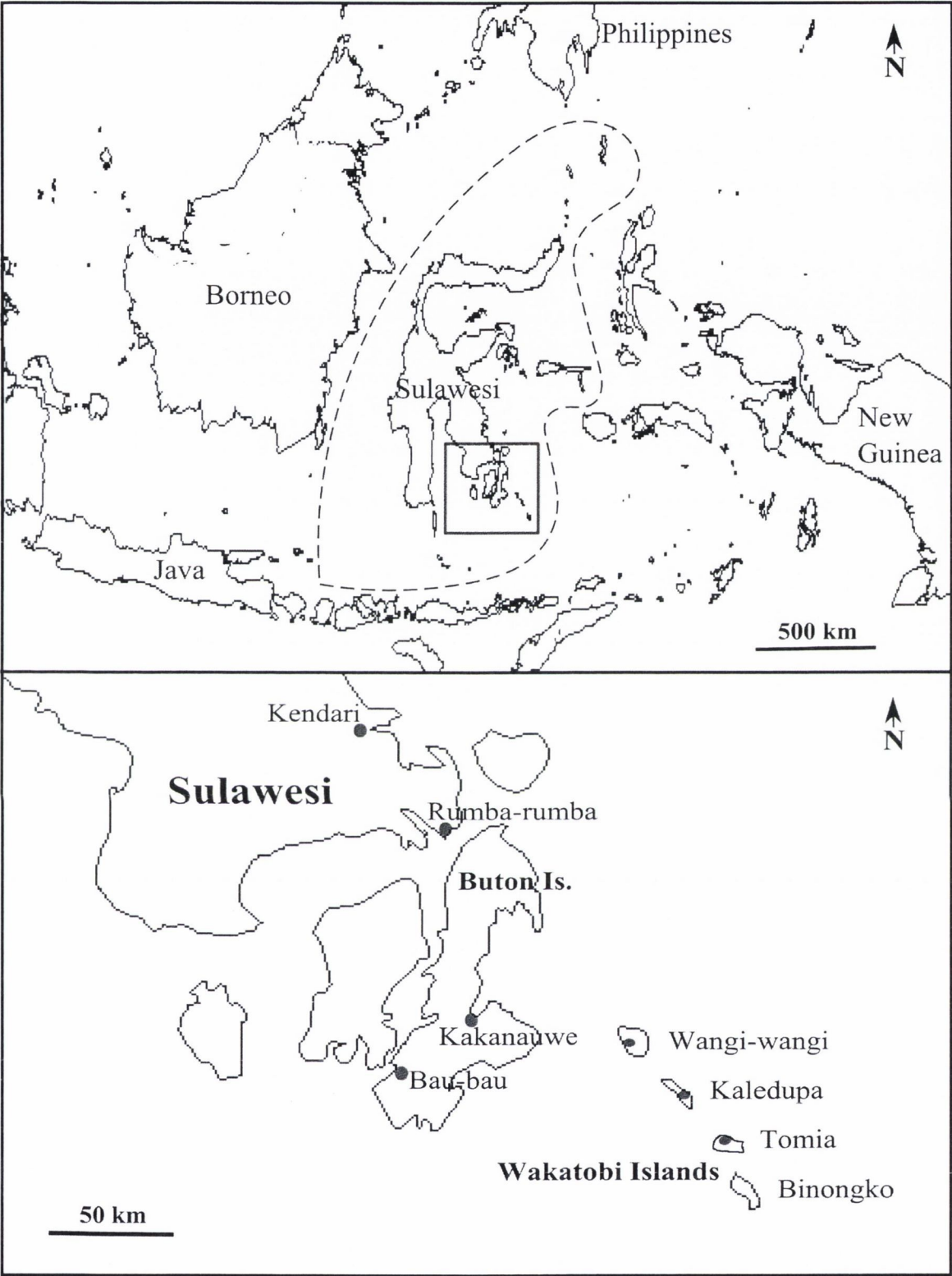


Figure 2.1: Map showing the Sulawesi region of Indonesia and study sites. Above: location and outline of the Sulawesi region of Indonesia (dashed lines) and the study area (square). Below: enlarged view of the study area showing peninsular south-east Sulawesi, Buton and the Wakatobi Islands; closed circles indicate sampling sites.

2.3.3 *Phylogenetic and Genetic Analyses*

A number of COI and ND3 sequences from *Dicaeum* and *Nectarinia* (outgroup) species were sourced from GenBank (Benson et al., 2013) to facilitate more robust phylogenetic analyses. The accession numbers of all sequences included can be seen in Table a.1. The outgroup for each phylogeny was composed of *Nectarinia* sunbirds (Nectariniidae), close relatives of the flowerpeckers (Dicaeidae) (Cheke and Mann, 2008a). Numerous sequences were included in the outgroup to generate a robust phylogenetic hypothesis (Luo et al., 2010). COI and ND3 sequences were aligned separately using ClustalW multiple alignment in BioEdit v.7.1.3.0 (Hall, 1999) and concatenated using Mesquite v.2.75 (Maddison and Maddison, 2011). While some partial tRNA fragments flanking the ND3 gene were sequenced, these sequences were not included in our analyses. Using MEGA v.5.0 (Tamura et al., 2011), a Hasegawa-Kishino-Yano model with five gamma categories and the presence of invariant sites (HKY+5 Γ +I) was chosen as the most suitable evolutionary model to infer the phylogeny of our combined genes. To avoid the over-parameterisation of our models we excluded invariant sites from our analyses. We carried out Bayesian phylogenetic inference of haplotypes using Mr. Bayes v.3.2.2 (Ronquist and Huelsenbeck, 2003). Our model parameters included a HKY substitution model, two independent runs consisting of four chains of five million generations, sampling every 1,000 generations and a burn-in of 25%. Convergence in runs was accepted when the average standard deviation in split frequencies reached 0.01 (Ronquist et al., 2012) and the effective sample size of model parameters exceeded 200 (Drummond et al., 2006). Maximum likelihood analysis was carried out in RaxML v.8 (Stamatakis, 2014), using a GTRCAT substitution model with five distinct gamma categories, partitioned by codon position and run for 1,000 rapid bootstrap replicates.

Using COI sequences, pairwise comparisons were carried out in MEGA v.5.0 (Tamura et al., 2011) to calculate maximum, minimum and mean uncorrected proportional genetic distances (p-distances) within and between Wakatobi and Sulawesi populations. These were then compared to known levels of between species genetic divergence (Hebert et al., 2004).

2.3.4 *Morphological Analyses*

We used two-way ANOVA models to compare populations from the Wakatobi archipelago to those on Sulawesi for each of the following morphological traits: wing length, weight,

tarsus length, tail length, skull length (total head minus bill length), and bill length and depth. All data were log10-normalized prior to analysis. As grey-sided flowerpeckers are sexually dimorphic (Cheke and Mann, 2008a), we included sex as a fixed effect in all models to control for this. We applied sequential Holm-Bonferroni corrections to correct for multiple comparisons (Holm, 1979). Our analyses were restricted to adult birds, as juvenile birds were netted only on the Wakatobi archipelago. All analyses were run in R v.3.0.1 (R Development Core Team, 2013).

2.4 Results

2.4.1 *Phylogenetic and Genetic Analyses*

Results from our maximum likelihood and Bayesian analyses of concatenated COI and ND3 haplotypes (Figure 2.2) described the Wakatobi and Sulawesi populations of grey-sided flowerpecker as reciprocally monophyletic, comprising two distinct evolutionary lineages. This split between Wakatobi and Sulawesi populations was strongly supported in both our maximum likelihood and Bayesian analyses (see Figure 2.2). Wakatobi and Sulawesi populations shared none of the same COI or ND3 haplotypes, suggesting the two populations were reproductively isolated. Furthermore, the branch lengths for Sulawesi populations were much longer than those of Wakatobi populations. We refrained from collapsing lineages with poor bootstrap (<50%) or Bayesian support (<0.95 posterior probability) to allow for the visualisation and comparison of divergent lineages within current taxonomic species. However, when we did collapse lineages with poor bootstrap support, Wakatobi and Sulawesi populations remained reciprocally monophyletic. Genetic divergence between the Wakatobi and Sulawesi populations for COI ranged from 2.53-2.83% and mean divergence within populations was 0.26%. Within the *Dicaeum* genus (ingroup), 159/351 (45.3%) of sites were variable and 118/351 (33.6%) sites were parsimony-informative for ND3, while 174/651 (26.8%) and 126/651 (19.4%) of sites were variable parsimony-informative for COI, respectively.

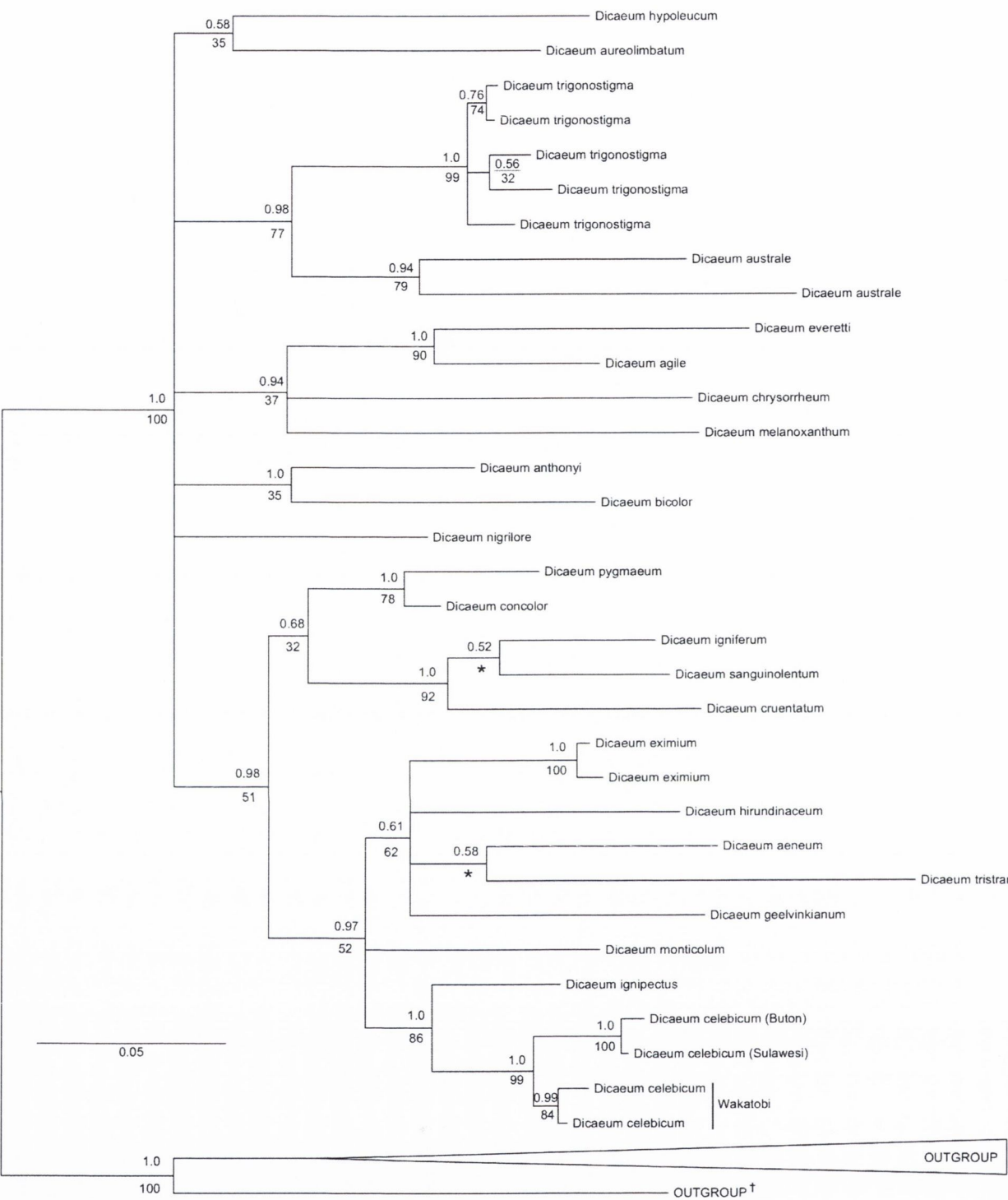


Figure 2.2: Bayesian consensus tree for concatenated COI and ND3 haplotypes, showing Bayesian posterior probabilities (above) and bootstrap values from our maximum likelihood analysis (below) for each node, with outgroup lineages collapsed. Nodes not present in our maximum likelihood analysis are marked with an asterisk. The accession numbers for all sequences included are given in Table a.1. The upper Wakatobi lineage contain samples from Wangi-wangi, Kaledupa and Hoga while the lower lineage contains samples from only Tomia. † This outgroup branch represents *Nectarinia sperata* which was monophyletic with respect to the rest of the outgroup and whose position with the genus remains uncertain (Cheke and Mann, 2008b).

Table 2.1: Morphological comparisons of adult male and female birds between Sulawesi and Wakatobi populations showing the mean and standard error for each character.

Trait:	Sulawesi ♂ (N = 17-20)	Sulawesi ♀ (N = 9)	Wakatobi ♂ (N = 9-16)	Wakatobi ♀ (N = 7)
Wing length	47.6 ±1.5	44.3 ±1.3	52.3 ±1.1	48.8 ±1.5
Weight	5.8 ±0.4	5 ±0.4	7 ±0.4	7.2 ±0.8
Bill length	11 ±0.4	10.8 ±0.6	13.1 ±0.5	12.8 ±0.4
Bill depth	2.7 ±0.1	2.6 ±0.2	2.8 ±0.2	2.8 ±0.2
Skull length	11.9 ±0.4	11.4 ±0.6	13.2 ±0.9	12.7 ±0.3
Tarsus length	11.2 ±0.5	10.7 ±0.5	12.8 ±0.4	12.6 ±0.4
Tail length	24 ±1	21.8 ±1	27.2 ±0.8	26.1 ±0.9

2.4.2 *Morphological Analyses*

Birds from the Wakatobi islands had significantly longer wings ($F_{1,49} = 152.76$, $p < 0.001$), bills that were longer ($F_{1,47} = 201.67$, $p < 0.001$) and deeper ($F_{1,39} = 17.02$, $p < 0.001$), longer tarsi ($F_{1,44} = 151.31$, $p < 0.001$), longer skulls ($F_{1,45} = 58.95$, $p < 0.001$), longer tails ($F_{1,39} = 130.12$, $p < 0.001$) and were heavier ($F_{1,48} = 136.93$, $p < 0.001$) than birds from mainland Sulawesi. These results control for the effects of sex and multiple testing. Morphological data are summarized in Table 2.1. Additionally, we observed the same differences in plumage between populations as originally described by Hartert (1903). Males from the Wakatobi archipelago have distinct steel-blue upperparts with a purplish gloss, compared to the dark purple upperparts of males from Sulawesi. The red colouration extends farther down the breast of Wakatobi males and they have lighter, greyer flanks and abdomen compared to Sulawesi males (see Figure 2.3). Consistent with other sources (Hartert, 1903; Cheke and Mann, 2008a), we noted no major plumage differences between females from Wakatobi and Sulawesi populations (see Figure 2.3).



Figure 2.3: A comparison of overall plumage characteristics between male (top row) and female (bottom row) flowerpeckers from mainland Sulawesi (left column) and the Wakatobi archipelago (right column). Photo credits: SBAK (top left) and DJK and NMM (all others).

2.5 Discussion

Our combined results from genetic, phylogenetic and morphological analyses, identify the Wakatobi populations of grey-sided flowerpecker as a distinct species, separate from the populations of mainland Sulawesi. Wakatobi and Sulawesi populations share no COI or ND3 haplotypes; this is one of the simplest tests of species identification using DNA barcodes (Hebert et al., 2004; Tavares and Baker, 2008). This also suggests that there is no gene flow between the populations and that they are reproductively isolated from one another, a defining criterion of species under the biological species concept (Mayr, 1942). The level of divergence in COI sequences between Wakatobi and Sulawesi populations (2.53-2.83%) is within the range of divergence between species (2.7%) observed by Hebert et al. (2004)

in a landmark study of over 260 North American bird species. Considering this, we believe the extent of genetic divergence between the two populations represents a separation at the species level.

Our morphological results support Hartert's (1903) original findings that Wakatobi populations are significantly larger than those on Sulawesi for various traits. Furthermore, this morphological divergence is suggestive of major ecological differences between these populations. The increases in tarsus length and bill size (length and depth) seen in Wakatobi populations are associated with changes in foraging and feeding ecology, respectively, both of which are suggestive of niche expansion (Grant, 1968; Scott et al., 2003). The observed increases in body size (which can be indexed from wing length and weight) may be due to reduced interspecific competition, increased intraspecific competition (with comparatively higher population densities) and/or variation in energetic constraints and physiological optimisation (Grant, 1968; Clegg and Owens, 2002; Robinson-Wolrath and Owens, 2003; Scott et al., 2003). These results suggest that despite being separated by only 27 km, there are likely to be major differences in the selective pressures acting upon the populations of small passerines on mainland Sulawesi and the Wakatobi archipelago. Behavioural and ecological studies of both Wakatobi and Sulawesi populations would help provide further insight into these suggested ecological differences.

Phylogenetic analyses of COI and ND3 sequences have provided useful corroborating evidence in the delimitation of other bird species, including other *Dicaeum* flowerpeckers (Nyári et al., 2009; Fjeldsa et al., 2010; Lohman et al., 2010). Some basal bootstrap and Bayesian support values from the inferred COI and ND3 phylogeny (Figure 2.2) are rather low and the overall structure of the phylogeny is thus not likely to accurately reflect broad relationships within the *Dicaeum* genus. A lack of sampling and gene sequencing of *Dicaeum* species is likely to be a contributing factor in this regard. Interestingly, the difference in branch lengths between Wakatobi and Sulawesi populations suggest Sulawesi lineages have experienced more genetic change since the populations split. Furthermore, the branch lengths separating Wakatobi and Sulawesi lineages are similar to those separating *D. trigonostigma* populations, a lineage which is known to contain currently undescribed species (Lohman et al., 2010).

When taken independently, our genetic, phylogenetic and morphological results provide limited evidence of divergence, but when combined they allow robust conclusions to

be drawn regarding species delimitation (e.g. Alström et al., 2008; McKay et al., 2010; Melo et al., 2010). Therefore, in consideration of our findings, previously described plumage differences (Cheke and Mann, 2008a) and a lack of justification for the departure from Hartert's original species description (Mayr and Amadon, 1947), we advocate the reclassification of the Wakatobi populations to their original status as *Dicaeum kuehni* (Hartert, 1903), a species endemic to the Wakatobi archipelago. We propose the vernacular name 'Wakatobi Flowerpecker' for *D. kuehni*. The recognition of *D. kuehni* as an endemic species will require BirdLife International to reassess their current categorisation of the Wakatobi archipelago as an Important Bird Area (ID184) (BirdLife International, 2013b). The Wakatobi currently hosts five endemic subspecies (Kelly and Marples, 2010), two of which were also originally described as species by Hartert (1903); therefore, in light of our findings, we believe these populations warrant re-examination. Furthermore, the Banggai and Sula, Sangihe and Talaud islands each support an endemic subspecies of *D. celebicum*, all of which deserve re-assessment using the same integrative approach as this study. Our study provides further evidence of unrecognised diversity and 'over-lumping' within the Sulawesi and Asian avifauna. Considering this, as well as the paucity of research, lack of multi-disciplinary studies of avian diversity and the complex geological history of the Sulawesi region, there are likely to be many more taxonomic relationships to be resolved and species to be described.

chapter 3

Incipient speciation of an island songbird lineage separated by a narrow oceanic barrier

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Author contributions: SBAK, DJK and NMM conceived and designed the project. SBAK, DJK, AB, KA and NMM performed the field and lab work. SBAK analysed the data. SBAK wrote the manuscript with input from DJK and NMM.

Status: This manuscript is being prepared for publication. Target journals include Royal Society Open Science, Ibis and the Journal of Avian Biology.

3.1 Abstract

An understanding of the process of speciation is central to evolutionary theory. Examples of incipient speciation, the divergence of evolutionarily distinct lineages, provide ideal opportunities to learn more about the mechanisms that drive speciation. Here we describe a case of incipient speciation within the olive-backed sunbird (*Cinnyris jugularis*). Olive-backed sunbirds from the Wakatobi Islands were originally described as an endemic species (*C. infrenata*, Hartert, 1903), but were later reclassified as *C. j. infrenatus*, a subspecies of olive-backed sunbird. Combining estimates of genetic, phylogenetic and phenotypic divergence, we reassessed the taxonomic status of the Wakatobi populations. Our results identify the Wakatobi populations as an incipient species, being genetically and phenotypically diagnosable from neighbouring Sulawesi populations but not genetically divergent to an extent that warrants recognition as a distinct species. We therefore support the current classification of Wakatobi populations as a subspecies of the olive-backed sunbird, *C. j. infrenatus*. We highlight the potential opportunity that Wakatobi populations, as an incipient species, present to study and further understand the patterns and processes of speciation. Furthermore, our results support claims of unrecognised intraspecific diversity within *C. jugularis* and raise interesting questions regarding the timing and pace of dispersal of *C. jugularis* east from Wallacea.

3.2 Introduction

Speciation as a result of geographic isolation, known as allopatric speciation, is one of the most common forms of speciation, particularly among island organisms as their separation promotes adaptive divergence and reproductive isolation (Price, 2008). Thus, empirical studies of island flora and fauna have come to form the basis to speciation research. Speciation is predominantly a gradual process driven by a complex array of mechanisms, progressing from initial adaptive divergence or genetic drift to assortative mating and, eventually, reproductive isolation. Therefore, present-day examples of any intermediate stages of speciation, termed incipient speciation, provide invaluable opportunities to further understand and describe the mechanisms driving speciation (Grant and Grant, 2008). Modern, integrative taxonomy, combining data on the physical and genetic make-up of organisms (Will et al., 2005), aids in the identification of incipient species and their divergent properties. This contemporary tool set allows researchers to investigate complex aspects of speciation, such as whether phenotypic adaptations have a genetic basis or are simply plastic responses to environmental stimuli (e.g. Greenberg et al., 2003), and whether phenotypic divergence has been driven by random genetic drift or other processes (e.g. Clegg et al., 2002b). Integrative analyses are also uncovering previously unrecognised species diversity (e.g. Lohman et al., 2010), providing new information on modes and rates of diversification (Moyle et al., 2009), and resolving taxonomic relationships among poorly studied taxa, such as the avifauna of Asia (BirdLife International, 2013a).

The olive-backed sunbird (*Cinnyris jugularis*, Linnaeus, 1766) is a small, sexually dimorphic passerine bird that can be found from Myanmar, Thailand and southern China, south through peninsular Malaysia, the Philippines and Indonesia to New Guinea, Australia and the Solomon Islands (Cheke and Mann, 2008b). There are currently 22 olive-backed sunbird (OBSB) subspecies recognised, among which there is extensive geographic variation in plumage, with males (all of which possess iridescent blackish feathering on the chin, throat and breast) showing the greatest variation in plumage types. Variation in male plumage ranges from the nominate subspecies *C. j. jugularis* which is olive-green above and bright yellow below, to *C. j. rhizophorae* which has distinct black and maroon breast-bands among otherwise greyish-white underparts, and *C. j. teysmanni* which has black and maroon breast-bands with blackish-purple underparts. Females also show geographical

plumage variation but to a lesser degree (see Cheke and Mann, 2008b).

There is a suggestion that the OBSB may, in fact, be made up of two separate species with surprising ranges. Populations from continental Asia, the Philippines, Greater and Lesser Sundas, Sulawesi, parts of the Moluccas, New Guinea, Bismarck Archipelago, Solomon Islands and Australia are suspected to comprise one species while the other is comprised of all remaining populations (i.e. those populations on small islands of the Lesser Sundas, Moluccas and Mamberamo region of northern New Guinea) (see Cheke and Mann, 2008b). However, recent genetic analyses by Lohman et al. (2010) suggest that the two Philippine subspecies, *C. j. jugularis* and *C. j. obscurior*, represent a distinct species, endemic to the Philippines. Using COI (cytochrome c oxidase subunit I) gene sequences, Lohman et al. (2010) calculated the lowest level of divergence between Philippine and non-Philippine OBSB populations as 3.2%, with the maximum divergence between all sequenced OBSB populations reaching 6.1%. These reported levels of COI divergence are above typical levels observed between closely-related bird species (2.7%; Hebert et al., 2004). Although evidence of genetic divergence alone is insufficient for species delimitation and description, it is indicative of currently unrecognised species diversity in *C. jugularis*. In consideration of these findings, *C. jugularis* may, in fact, be comprised of at least two to three different species, if not more. This may not be surprising considering the vast geographic range and large number of islands colonised by *C. jugularis*.

The OBSB present on the Wakatobi Islands, currently classified as *C. j. infrenatus*, were originally described as a separate species (*C. infrenata*, Hartert, 1903), distinct from neighbouring OBSB populations on Sulawesi (*C. j. plateni*) and the Moluccas (*C. j. frenatus*). Hartert (1903) relied solely on comparisons of plumage and morphology from museum specimens in his original description. Wakatobi birds were differentiated as males lacked the yellow supercilium and malar stripes of Sulawesi and Moluccan populations and exhibited contrastingly darker upperparts and deeper yellow underparts which were often tinged with orange (Hartert, 1903). Wakatobi males were also determined to be larger than those from the Moluccas. Wakatobi females were differentiated as being larger than those on Sulawesi, with darker overall plumage and a highly reduced and duller supercilium (Hartert, 1903; Kelly and Marples, 2011). However, Hartert (1920) later reclassified the Wakatobi species *C. infrenata* as a subspecies of the OBSB, *C. jugularis infrenatus*. While there is no outline of the justification for this reclassification, it probably coincided with Hartert's increased application of trinomial nomenclature (see Stresemann,

1975). Interestingly, when Hartert subsumed the Wakatobi populations into *C. jugularis*, he refrained from subsuming the Wakatobi flowerpecker (*Dicaeum kuehni*; see Chapter 2) into the grey-sided flowerpecker (*D. celebicum*) from Sulawesi. Perhaps this suggests Hartert had less confidence in the separation of Wakatobi sunbird populations as a distinct species compared to the separation of the Wakatobi flowerpecker (Kelly et al., 2014).

More than 100 years on from Hartert's original description (Hartert, 1903), we combined genetic, phylogenetic and phenotypic analyses for a modern, rigorous assessment of the taxonomic status of OBSB populations from the Wakatobi Islands. We compared Wakatobi populations to their closest neighbouring populations on Sulawesi (*C. j. plateni*). While these populations are separated by only 27 km of ocean (see Figure 1.1), the 1500-2000 m ocean depths in this divide have kept mainland Sulawesi and the Wakatobi Islands physically disconnected (Kaye, 1989; Milsom et al., 1999), even with the c. 125 m drop in sea level at the last glacial maxima (Fairbanks, 1989). This relatively narrow oceanic barrier may have prevented gene flow between Sulawesi and Wakatobi OBSBs, thus promoting divergence, as we observed among flowerpecker populations (Kelly et al., 2014). Our results describe the Wakatobi populations as an incipient species, divergent from mainland populations but not currently warranting recognition as a separate species. Our genetic and phylogenetic analyses provide further evidence of currently unrecognised species diversity within *C. jugularis* and raise questions regarding the dispersal of this taxon east from Sulawesi.

3.3 Methods

3.3.1 Sampling

Sampling took place on peninsular South-east Sulawesi and the islands of Buton and Kabaena, and across the Wakatobi Islands (see Figure 1.1). Birds were mist-netted opportunistically during the months of July, August and September in various years between 1999 and 2012. Mist nets were erected before dawn in a variety of habitat types; chosen for their accessibility and suitability. After being assessed for age (adult or juvenile) and sex, the following morphometric measurements were taken for each netted bird: wing length (maximum chord), weight, bill length (to skull), bill depth (at distal end of nares), tail length (standard), tarsus length ('minimum') and total head length (head plus bill) (Redfern and Clark, 2001). Prior to release, a small cluster of contour feathers were plucked from the flank of each bird.

3.3.2 DNA Sequencing

DNA was extracted from feathers using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, California, USA). Extraction followed the manufacturer's protocol except for (i) the addition of 5 μ l of dithiothreitol (DTT), after the prescribed addition of 20 μ l of proteinase K, to improve keratin digestion and (ii) the addition of 150 μ l of Buffer AE instead of 200 μ l in the double elution of bound DNA from the spin column. A region of the COI gene, c. 672 bp in length, was amplified using barcoding primers Passer-F1 and Passer-R1 (Lohman et al., 2009). The complete mitochondrial gene ND3 (NADH dehydrogenase subunit 3; 351 bp) and fragments of the flanking tRNAs (tRNA-Gly and tRNA-Arg) were amplified using primers L10755-F and H11151-R (Chesser, 1999) which have proved successful in previous OBSB studies (Nyári et al., 2009; Fjeldsa et al., 2010). All polymerase chain reaction (PCR) amplifications were performed in 20 μ l solutions consisting of 9.3 μ l double-distilled H₂O, 0.4 μ l 10 mM deoxynucleoside triphosphates (dNTPs), 2 μ l 10x PCR reaction bufferF, 1.2 μ l 50 mM MgCl₂, 1 μ l 10 μ M forward primer, 1 μ l 10 μ M reverse primer, 0.1 μ l Taq polymerase and 5 μ l template DNA. All COI reactions were amplified under the following thermal cycler conditions: 4 min at 94 °C followed by 45 cycles of 1 min at 94 °C, 1.5 min at 58 °C and 1.5 min at 72 °C, finishing with 5 min at 72 °C (adapted from Kerr et al., 2007). Thermal cycling conditions for ND3 amplification were the same except the second step of this cycling was lowered from 58 °C to 55 °C (adapted from Chesser, 1999). The addition of DTT, the increase of DNA template in PCR reactions from 3 μ l to 5 μ l (and the associated decrease of H₂O in reactions from 11.3 μ l to 9.3 μ l), and the running of COI PCR cycles for 45 cycles were alterations of the original protocol used in Chapter 2 (Section 2.3.2) to maximise the successful extraction, amplification and sequencing of the relatively small tissue samples that feather tips present.

PCR products were then cleaned using ExoSAP protocol (Werle et al., 1994), cycle-sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, California, USA), subsequently cleaned with BigDye XTerminator Purification Kit (Applied Biosystems, California, USA) and sequenced in both directions on an Applied Biosystems 3130xl genetic analyzer.

3.3.3 Phylogenetic and Genetic Analyses

To gain a greater understanding of the genetic and phylogenetic diversity across the geographic range of *C. jugularis*, all available *C. jugularis* COI and ND3 sequences from

GenBank (Benson et al., 2013) were included in our analyses. To generate more robust phylogenetic trees (see Luo et al., 2010), all available COI and ND3 sequences from the sunbird genus *Cinnyris* and the flowerpecker genus *Dicaeum* (outgroup; close relatives of the sunbirds) were included in our analyses. As the *Cinnyris* genus is often subsumed in the genus *Nectarinia*, we included all available COI and ND3 sequences of *Nectarinia* species, as well as sequences from those other sunbird genera often subsumed in *Nectarinia* (i.e. *Chalcomitra*, *Cyanomitra* and *Leptocoma* sunbirds). The accession numbers of all sequences included can be seen in the Appendices, Table b.1.

Using all the available COI sequences for Sulawesi and Wakatobi populations, pairwise comparisons with 1,000 bootstrap replicates were carried out in MEGA v.5.0 (Tamura et al., 2011) to calculate maximum, minimum and mean uncorrected proportional genetic distances (p-distances) within and between populations. Furthermore, using all available OBSB COI sequences, we used pairwise comparisons to calculate mean uncorrected p-distances between Wakatobi and Sulawesi populations and other OBSB subspecies. These distances were then compared to known levels of between species genetic divergence (see Hebert et al., 2004).

COI and ND3 sequences were aligned separately using ClustalW multiple alignment in BioEdit v.7.1.3.0 (Hall, 1999) and concatenated using Mesquite v.2.75 (Maddison and Maddison, 2011). Using MEGA v.5.0 (Tamura et al., 2011), a Hasegawa-Kishino-Yano model with five gamma categories (HKY+5 Γ +I) was chosen as the most suitable evolutionary model to infer the phylogeny of our combined genes. To avoid the over-parameterisation of our models we excluded invariant sites from our analyses. Maximum likelihood phylogenetic inference was carried out in RaxML v.8 (Stamatakis, 2014), using a GTRCAT substitution model with five distinct gamma categories, partitioned by codon position and run for 1,000 rapid bootstrap replicates. We carried out Bayesian phylogenetic inference of haplotypes using MrBayes v.3.2.2 (Ronquist and Huelsenbeck, 2003). Our model parameters included a HKY substitution model, two independent runs consisting of four chains of 19 million generations, sampling every 1,000 generations and a burn-in of 25%. Convergence in runs was accepted when the average standard deviation in split frequencies reached 0.01 (Ronquist et al., 2012) and the effective sample size of model parameters exceeded 200 (Drummond et al., 2006). The resulting ML and Bayesian phylogenetic trees were rooted at the outgroup.

3.3.4 *Morphological Analyses*

As many avian morphological traits, such as wing length and weight, are highly correlated (Rising and Somers, 1989), we used principal component analysis (PCA) to identify high correlation among traits and determine the greatest sources of variation in our data. PCA produces independent variables, known as principal components, which are orthogonal axes created by linear combinations of the original morphological variables and can loosely be interpreted as measures of size and shape when using avian morphological data (Rising and Somers, 1989; Ricklefs, 2012). Each principal component (PC) explains a certain percentage of variation in the data, with the first PC (PC1) explaining the greatest variation and each subsequent PC explaining a lesser percentage. Each PC is weighted (or loaded) to a varying degree by some or all of the original variables. As PCA cannot deal with missing values, the inclusion of all seven morphological traits in our analyses would have greatly reduced our sample size. Therefore, in order to maximise statistical power, we excluded those traits with a high proportion of missing values (i.e. bill depth, tail length and tarsus length) and ran PCA using data on wing length, weight, bill length and skull length. Gaps in our dataset arose as a result of: (i) the release of individual birds showing signs of heat stress or other indicators of poor body condition before they were processed completely; and (ii) a morphological sampling protocol that was continually developed. As our morphological variables differed in their scales, we scaled each variable prior to PCA by standardizing each measurement relative to the mean and standard deviation of that variable.

Taking the principal components generated from our PCA, we used multi-factorial analysis of variance (ANOVA) models to determine whether there were any significant morphological differences between Wakatobi and Sulawesi populations. Furthermore, we used multi-factorial ANOVAs of PCs to determine the extent of sexual dimorphism in each subspecies. We also ran post-hoc multi-factorial ANOVAs on PCs to determine whether there was any significant inter-island variation within each subspecies. In all analyses, we controlled for the effects of variation in processor accuracy (i.e. differences between individuals measuring traits), the age of the birds (as we included both adult and juvenile birds) and the sex of each bird (as we know OBSB are sexually dimorphic) where relevant. Thus, in our analyses, we only included those birds whose sex was determined. We calculated effect size (ES) statistics, using Cohen's unbiased D (see Nakagawa and Cuthill,

2007), for each of our comparisons to estimate the magnitude of any differences. All analyses were carried out in R v.3.0.2 (R Development Core Team, 2013).

3.4 Results

3.4.1 Sampling

In total, 284 OBSB (170 males, 109 females and five birds of undetermined sex) were netted; 79 individuals of the Sulawesi subspecies *C. j. plateni* (24 individuals from peninsular South-east Sulawesi, 19 from Buton and 32 from Kabaena) and 205 individuals of the Wakatobi subspecies *C. j. infrenatus* (28 from Wangi-wangi, 16 from Oroho, 31 from Kaledupa, 54 from Hoga, 32 from Tomia, 20 from Lintea, 19 from Binongko and five from Runduma). Morphological measurements were taken for each of these birds; however, permit restrictions meant we were unable to take feather samples from all individuals.

3.4.2 DNA Sequencing

We successfully generated COI and ND3 sequences from 35 and 24 OBSB individuals, respectively. Of those 35 individuals sequenced for COI, 11 were of the Sulawesi subspecies *C. j. plateni* and 24 were of the Wakatobi subspecies *C. j. infrenatus*. Of the 24 individuals sequenced for ND3, 10 were of the Sulawesi subspecies *C. j. plateni* and 14 were of the Wakatobi subspecies *C. j. infrenatus*.

3.4.3 Phylogenetic and Genetic Analyses

Genetic divergence between Wakatobi and Sulawesi populations for COI ranged from 1.29-2.03% with a mean divergence (plus or minus standard error) of $1.63 \pm 0.46\%$. Genetic divergence among Wakatobi populations ranged from 0-0.92% with a mean of $0.33 \pm 0.16\%$ while divergence among Sulawesi populations ranged from 0-1.48% with a mean of $0.211 \pm 0.07\%$. Estimates of mean genetic divergence for COI between Wakatobi and Sulawesi and other *C. jugularis* subspecies are summarised in Table 3.1.

Both our maximum likelihood (ML) and Bayesian analyses of concatenated COI and ND3 gene sequences describe the Wakatobi populations of OBSB as a distinct, monophyletic group with strong support. The complete ML tree (Figure 3.1) and Bayesian tree (Figure 3.2) show the inferred phylogenetic relationships between the monophyletic *C. jugularis* and other *Cinnyris*, *Nectarinia*, *Chalcomitra*, *Cyanomitra* and *Leptocoma* sunbird species,

Table 3.1: Mean estimates of COI divergence ($\pm$ standard error) between Wakatobi and Sulawesi and other OBSB subspecies. Sample sizes refer to number of COI sequences per subspecies available (including those generated in this study and those available on GenBank).

Subspecies compared:	<i>C. j. infrenatus</i> (Wakatobi; N = 24)	<i>C. j. plateni</i> (Sulawesi; N = 14)
<i>C. j. plateni</i> (Sulawesi ; N = 14)	1.63 $\pm$ 0.46%	-
<i>C. j. flavigastrea</i> (Solomon Islands ; N = 5)	1.89 $\pm$ 0.52%	0.3 $\pm$ 0.09%
<i>C. j. jugularis</i> (Cebu and Sibuyan, Philippines ; N = 7)	4.85 $\pm$ 0.94%	5.49 $\pm$ 0.99%
<i>C. j. obscurior</i> (Luzon, Philippines ; N = 3)	4.82 $\pm$ 0.93%	5.45 $\pm$ 0.98%
<i>C. j. ornatus</i> (Malaysia, Singapore and Sumatra; N = 3)	4.75 $\pm$ 0.9%	4.63 $\pm$ 0.89%

while subsets of the ML (Figure 3.3A) and Bayesian (Figure 3.3B) trees show intraspecific, geographic variation in *C. jugularis*. Our ML analysis describes the Wakatobi populations as monophyletic (with 94% bootstrap support) with respect to Sulawesi populations, while Sulawesi populations are described as paraphyletic (with this clade including populations from the Wakatobi and Solomon Islands; see Figure 3.3A). The ML tree provides very low support for the separation of Sulawesi populations from those in the Solomon Islands and Australia (see Figure 3.3A). Our Bayesian analysis shows a similar pattern, providing strong support (posterior probability of 1) for monophyly of the Wakatobi populations and no strong separation of populations from Sulawesi, the Solomon Island and Australia (see Figure 3.3B). In both our ML and Bayesian trees, there are suggestions of two separate lineages with the Wakatobi Islands but the split of these lineages is not strongly supported (see Figure 3.3). Furthermore, both our ML and Bayesian trees describe an OBSB lineage comprising Philippine populations and another lineage comprising populations from Singapore, Sumatra and continental Malaysia. Both lineages are separated from one another and from other OBSB populations with high support values (see Figure 3.3).

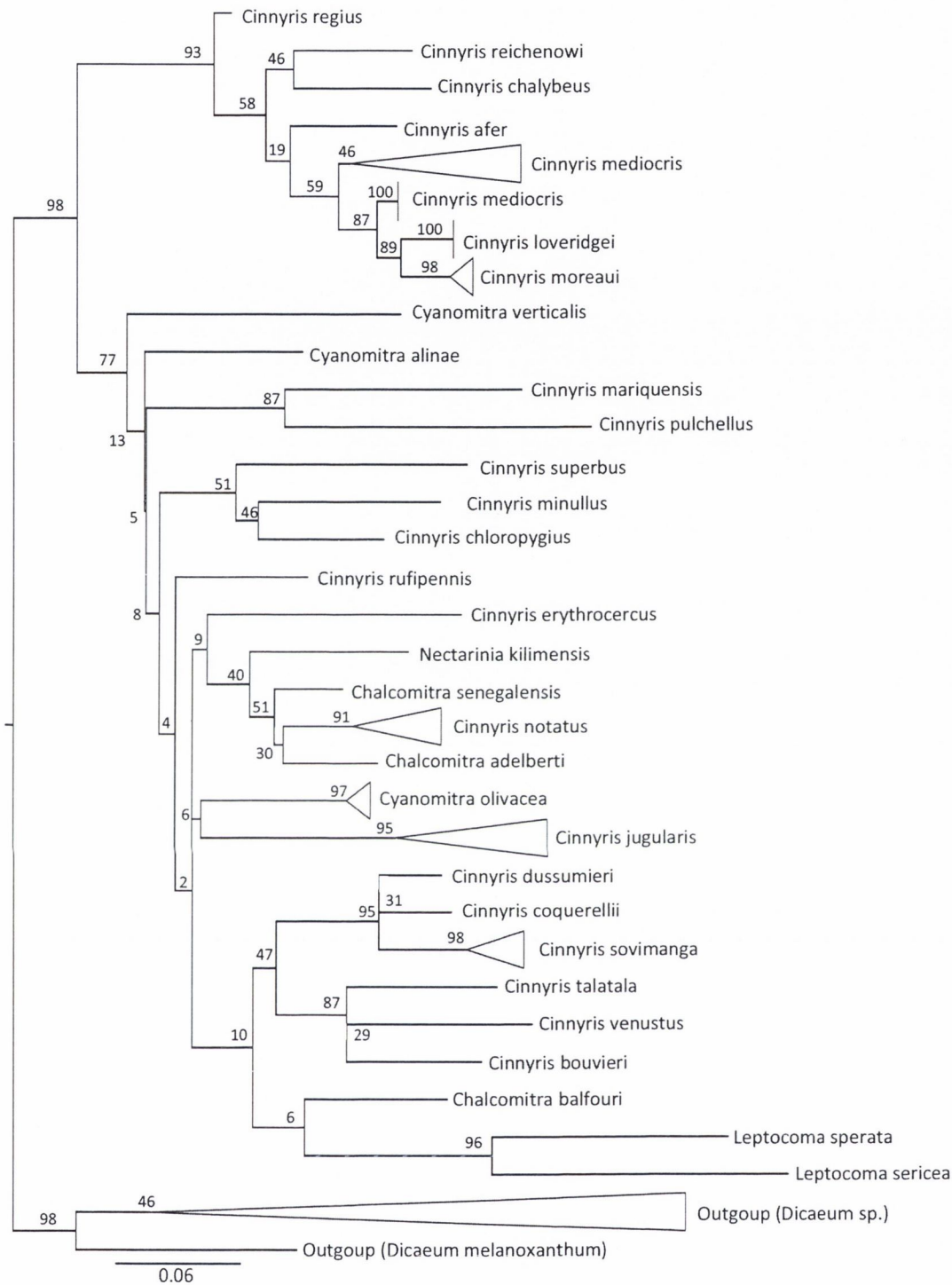


Figure 3.1: Maximum likelihood tree for concatenated COI and ND3 sequences showing species-level diversity with bootstrap support values for each node and outgroup lineages collapsed. Note: *Dicaeum melanoxanthum* is often placed within its own, separate genus, *Pachyglossa* (Cheke and Mann, 2008a).

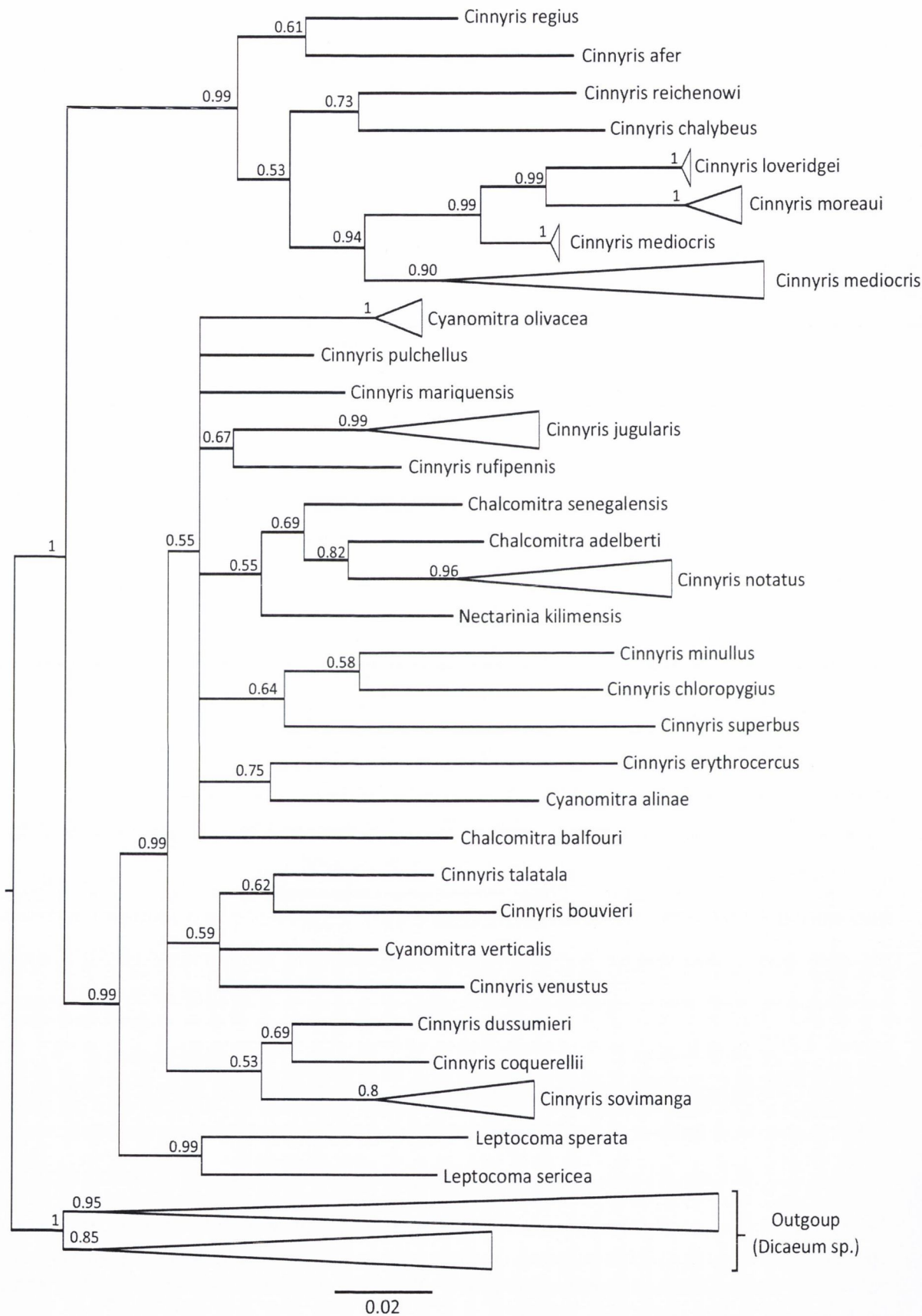


Figure 3.2: Bayesian consensus tree for concatenated COI and ND3 sequences showing species-level diversity with posterior probabilities for each node and outgroup lineages collapsed.

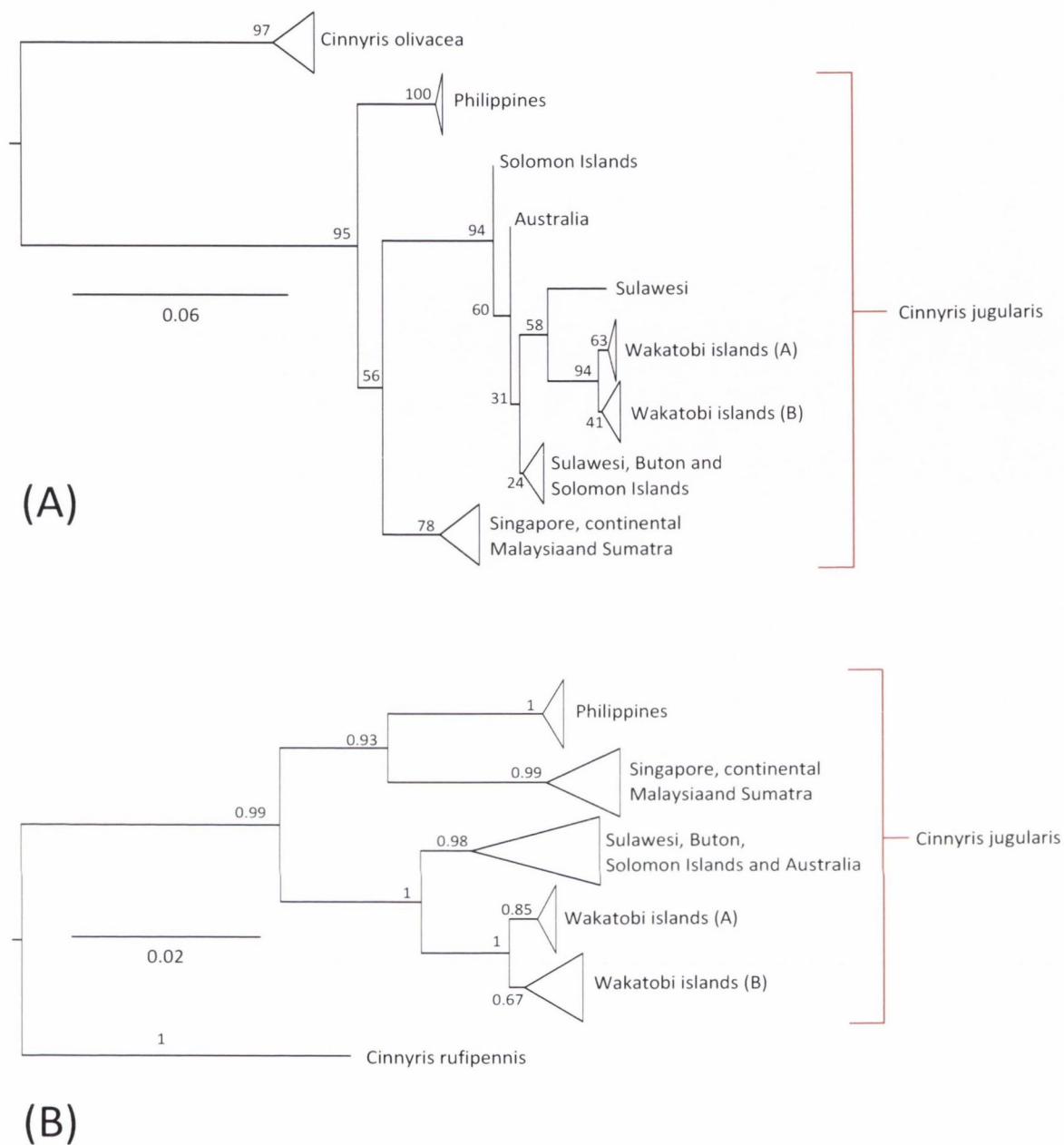


Figure 3.3: A subset of (A) maximum likelihood and (B) Bayesian consensus trees, showing intraspecific, geographical variation for *C. jugularis*. Bootstrap support values and posterior probabilities are shown at each node in (A) and (B), respectively. The clades labelled 'Wakatobi islands (A)' includes individuals from Hoga, Tomia, Binongko and Runduma while 'Wakatobi islands (B)' clades include individuals from Wangi-wangi, Oroho, Kaledupa, Hoga, Tomia, Lentea and Runduma.

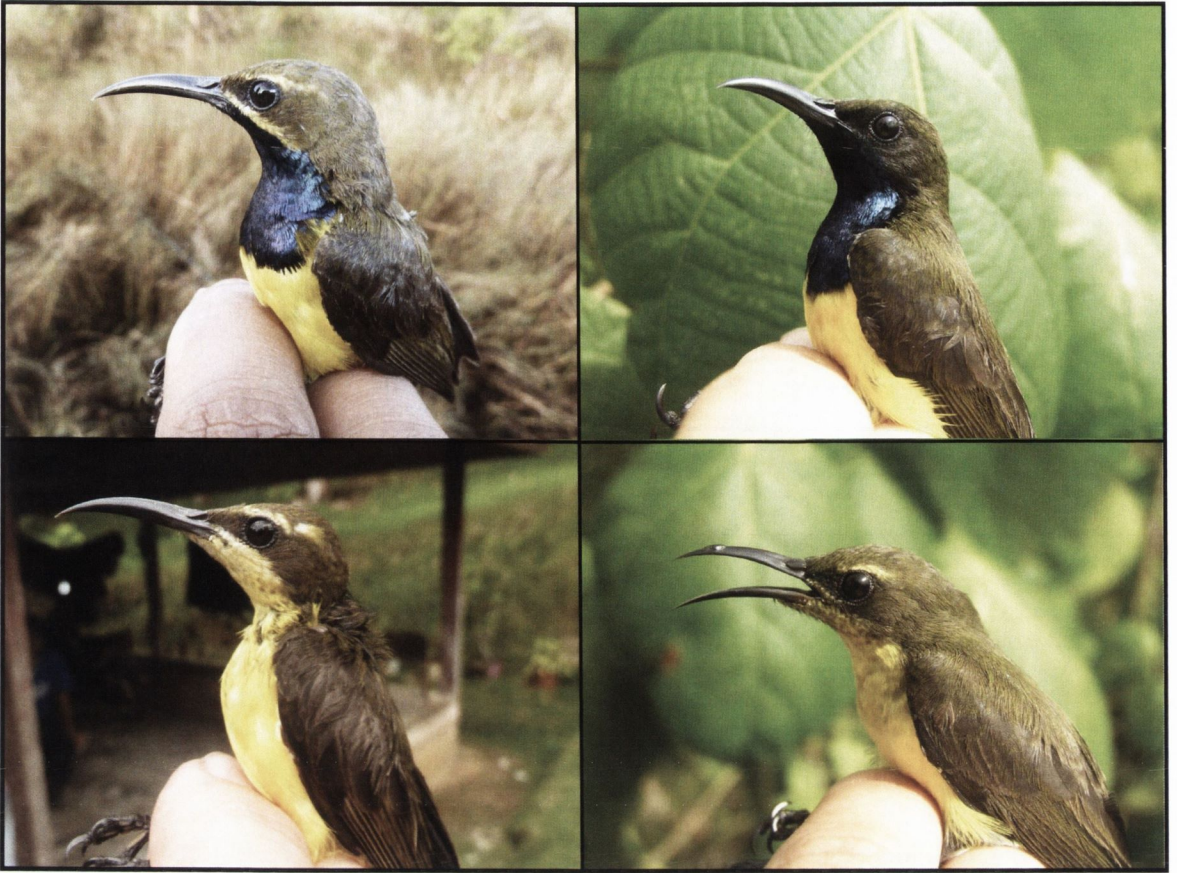


Figure 3.4: A comparison of overall plumage characteristics between male (top row) and female (bottom row) olive-backed sunbirds from mainland Sulawesi (left column) and the Wakatobi archipelago (right column). Photo credits: DJK and NMM.

3.4.4 *Morphological Analyses*

The first two principal components from our PCA explained 84.6% of the morphological variation seen within Wakatobi and Sulawesi populations. PC1 explained 51.1% of the variation in the data and gave an overall indication of body size, being largely weighted by wing length, weight and bill length (PC1 loading coefficients: wing length: -0.61; weight: -0.61; bill length: -0.49; skull length: -0.10). PC2 explained 33.5% of the variance in the data and was highly weighted by skull and bill length, giving a combined estimate of the ratio of bill length to skull length (PC2 loading coefficients: skull length: -0.82; bill length: 0.54; wing length: -0.16; weight: -0.13). The variation between Wakatobi and Sulawesi populations around these axes is shown in Figure 3.5. After controlling for the effects of variation in processor and age and sex of birds, we found Wakatobi birds were significantly smaller than those from Sulawesi (PC1, body size: ANOVA: $F_{1,242} = 11.37$; $p < 0.001$; ES = -0.33) but found no significant change in the ratio of bill length to skull length between populations (PC2: ANOVA: $F_{1,242} = 0.2$; $p = 0.65$; ES = -0.45). Sulawesi populations showed high sexual dimorphism in body size (PC1: ANOVA: $F_{1,49} = 7.91$; $p < 0.001$; ES = -1.86) but

not for bill to skull ratio (PC2: ANOVA: $F_{1,49} = 1.34$; $p = 0.25$; $ES = -0.31$). While Wakatobi populations showed a similar pattern for body size (PC1: ANOVA; $F_{1,189} = 362.41$; $p < 0.001$; $ES = -2.83$), there was some sexual dimorphism in bill to skull ratio (PC2: ANOVA; $F_{1,189} = 3.819$; $p = 0.05$; $ES = -0.1$). Wakatobi populations showed significant inter-island variation in body size ($F_{6,179} = 8.52$; $p < 0.001$) and bill length:skull length ($F_{6,179} = 6.90$; $p < 0.001$). Note: this inter-island variation is explored further in Chapter 5. We found no such morphological variation among Sulawesi populations (PC1: $F_{2,43} = 0.93$; $p = 0.40$; PC2: $F_{2,43} = 1.77$; $p = 0.18$). The morphological variation within and between populations (for non-transformed variables) is summarised in Table 3.2.

During our sampling, we noticed the same consistent differences in plumage between Wakatobi and Sulawesi populations as previously recorded (Hartert, 1903; Kelly and Marples, 2011). While the male birds from Sulawesi have characteristic yellow supercilial and malar stripes, Wakatobi males lack these features entirely, have generally darker upperparts and have a deeper yellow breast and abdomen which is regularly tinged with orange. While females from both populations look quite similar, Sulawesi females have strong yellow supercilial stripes which are greatly reduced and duller in Wakatobi females. These features are shown in Figure 3.4

Table 3.2: A summary of the morphological variation between males and females of the Wakatobi and Sulawesi populations for all morphological traits sampled. For each character and group, the mean $\pm$ standard error and sample size are shown. Values shown are from adult birds only and all traits are presented in units of millimetres, except weight which is presented in grams

Trait	Wakatobi ♂	Wakatobi ♀	Sulawesi ♂	Sulawesi ♀
Wing length	54.5 $\pm$ 0.1 (N = 113)	50.9 $\pm$ 0.1 (N = 78)	55.8 $\pm$ 0.2 (N = 44)	52.7 $\pm$ 0.3 (N = 24)
Weight	8.2 $\pm$ 0.1 (N = 113)	7.2 $\pm$ 0.1 (N = 77)	8.1 $\pm$ 0.1 (N = 43)	7.2 $\pm$ 0.1 (N = 22)
Bill length	21 $\pm$ 0.1 (N = 113)	20 $\pm$ 0.1 (N = 78)	21.5 $\pm$ 0.2 (N = 41)	20.5 $\pm$ 0.2 (N = 23)
Bill depth	2.5 $\pm$ 0.1 (N = 77)	2.4 $\pm$ 0.1 (N = 58)	2.6 $\pm$ 0.1 (N = 22)	2.4 $\pm$ 0.1 (N = 10)
Bill width	6.6 $\pm$ 0.1 (N = 3)	7.3 $\pm$ 0.4 (N = 4)	6.7 $\pm$ 0.2 (N = 10)	7 $\pm$ 0.1 (N = 5)
Skull length	12.9 $\pm$ 0.1 (N = 110)	12.8 $\pm$ 0.1 (N = 74)	13.3 $\pm$ (0.1) (N = 31)	12.9 $\pm$ 0.2 (N = 18)
Tarsus length	15 $\pm$ 0.1 (N = 107)	14.3 $\pm$ 0.1 (N = 77)	14.1 $\pm$ 0.1 (N = 25)	13.9 $\pm$ 0.1 (N = 15)
Tail length	37.4 $\pm$ 0.1 (N = 77)	33.3 $\pm$ 0.2 (N = 58)	35.6 $\pm$ 0.3 (N = 23)	33 $\pm$ 0.4 (N = 10)

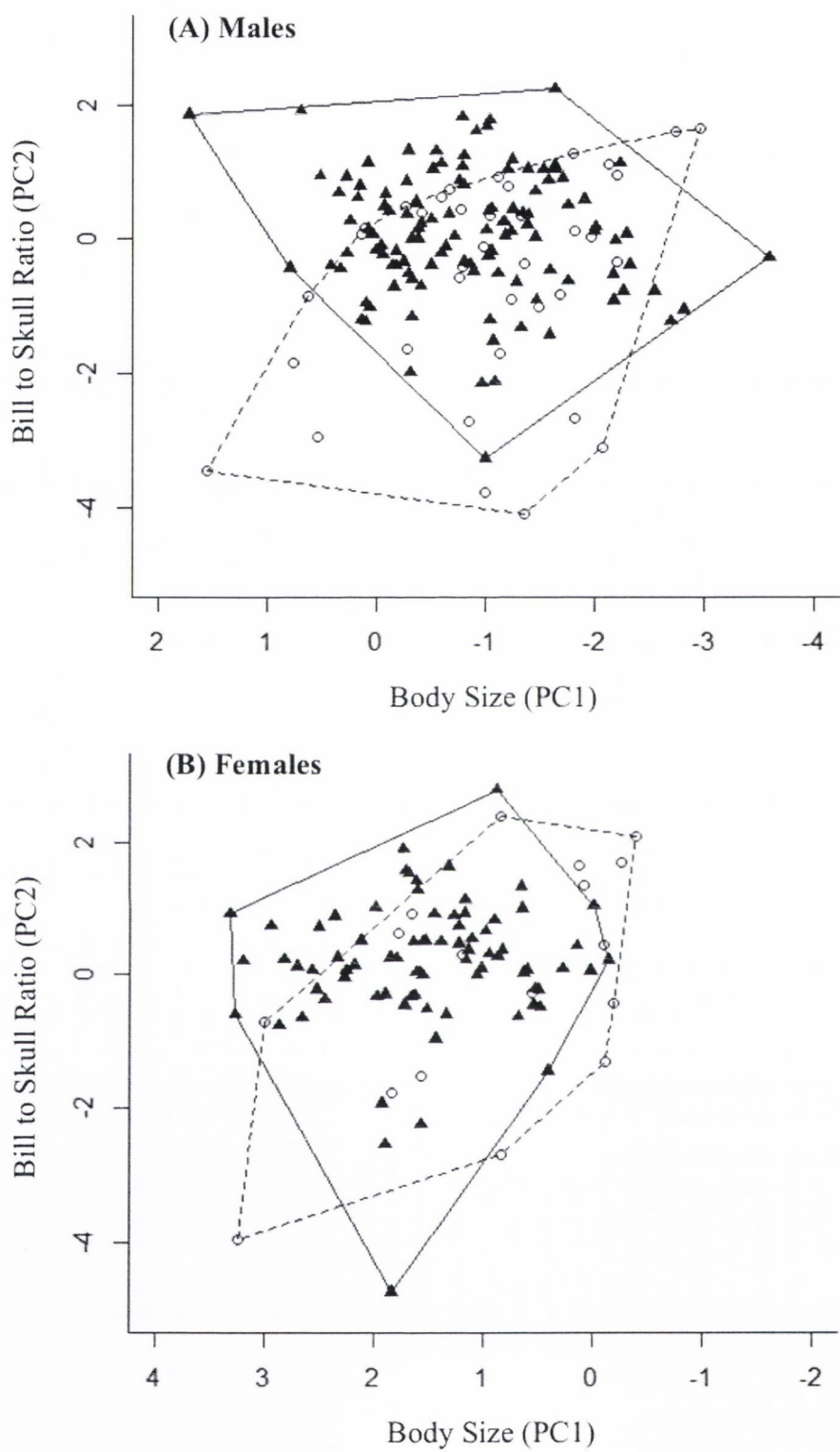


Figure 3.5: Comparison of body size and bill to skull ratio in (A) male and (B) female birds from Wakatobi (filled triangles, solid lines) and Sulawesi (open circles, dashed line) populations. Lines show convex hull for each population. Note: negative PC1 values correspond to larger body sizes.

3.5 Discussion

Our combined results from genetic, phylogenetic and morphological analyses identify the Wakatobi populations, *C. j. infrenatus*, as an incipient species, divergent from *C. j. plateni* on Sulawesi but not to such an extent to warrant recognition as a separate species. Our ML and Bayesian analyses determined that Wakatobi and Sulawesi populations share none of the same COI or ND3 haplotypes such that both lineages can be positively identified using DNA barcoding (Tavares and Baker, 2008). Furthermore, this suggests that Sulawesi and Wakatobi populations are not interbreeding and that the 27 km oceanic divide between populations forms a pre-zygotic barrier to reproduction. However, the level of divergence in COI sequences between populations (1.29-2.03%) is considerably lower than that observed between other closely-related bird species (Hebert et al., 2004; Kerr et al., 2007; Tavares and Baker, 2008). Other studies have shown that the minimum level of divergence between even closely-related species typically exceeds 10 times the mean level of divergence within species (see Hebert et al., 2004; Tavares and Baker, 2008). The minimum level of divergence between Sulawesi and Wakatobi populations was 1.29%. The mean level of divergence within populations was 0.27%. Thus, as the minimum level of divergence between Wakatobi and Sulawesi populations did not exceed 10 times the mean level of divergence within populations, this suggests they do not represent different species. This '10 times' value (2.7%) is identical to the value observed in a study of over 260 bird species (Hebert et al., 2004).

From our morphological comparisons we determined that Wakatobi birds were significantly smaller in body size than Sulawesi birds. We found no difference in the relative length of bill and skull between populations which suggests that skull length remained proportional to bill length in each population. While Hartert (1903) described the Wakatobi females as larger than those from Sulawesi, our results suggest the opposite and are likely to be more conclusive due to our larger sample size and wider sampling of morphological traits in live birds. Despite possessing diagnostic plumage and morphological traits, as well as diagnostic COI and ND3 barcodes, we do not consider the extent of divergence between Wakatobi and Sulawesi populations as indicative of a species-level difference. Preliminary research suggests there are differences in the acoustic signals of Wakatobi and Sulawesi populations (Naomi Harte, unpublished data). If Wakatobi and Sulawesi populations were seen to have distinct songs and calls that promote high assortative mating, this acoustic evidence, combined with the evidence presented in this study, could have a bearing on

their taxonomic classification (Tobias et al., 2010). Furthermore, this would provide an opportunity to further study the mechanisms that drive divergence in acoustic signals and further understand how such acoustic divergence can drive speciation.

The divergence of Wakatobi populations is likely to have been driven by a variety of processes, with small founder populations presenting initially low genetic and phenotypic diversity and therefore experiencing genetic bottlenecks, with further genetic and phenotypic differences accumulating as a result of increased adaptation to the local environment via mutations, recombination, genetic drift, directional selection and selective sweeps (Price, 2008; Stuessy et al., 2012). This divergence will increase with time (if gene flow between populations remains low or absent) and populations may eventually become recognized as distinct species (Brelsford and Irwin, 2009; Stuessy et al., 2012). In line with theoretical predictions that island populations experience reduced genetic diversity as a result of founder effects and small populations (Whittaker and Fernández-Palacios, 2007), we observed lower COI variation within Wakatobi populations (0-0.92%) compared to Sulawesi populations (0-1.48%). The reduced body size in Wakatobi birds deviates from the general trend of insular birds showing larger body sizes relative to their mainland counterparts (Meiri et al., 2011). The divergence in body size between Wakatobi and Sulawesi populations suggests there are differences in ecological and environmental pressures between allopatric sites. Phenotypic divergence in Wakatobi populations was likely to have been facilitated and promoted as a result of differences in: levels of predation and inter- and intra-specific competition for resources; feeding ecology; the diversity and quality of resources available; and energetic constraints and physiological optimisation (Grant, 1968; Clegg and Owens, 2002; Robinson-Wolrath and Owens, 2003; Scott et al., 2003; Beauchamp, 2010). The processes that drive divergence in body size are investigated further in Chapter 5.

Wakatobi and Sulawesi populations were also seen to show a similarly high degree of sexual dimorphism in body size which suggests levels of sexual selection are similar between Sulawesi and the Wakatobi Islands. We detected no sexual dimorphism in the ratio of bill length to skull length in either population. There was significant inter-island variation in both body size and bill length:skull length within Wakatobi populations, suggesting there were inter-island differences in the levels of inter- and intra-specific competition, resource diversity and availability, predation, etc. The patterns and drivers of morphological variation among Wakatobi populations are investigated in Chapter 5. We

detected no such variation among Sulawesi populations (i.e. peninsular Sulawesi, Buton and Kabaena); however, variation in bill length among these populations has previously been observed (Darren O'Connell, unpublished data).

Our ML and Bayesian phylogenetic analyses described populations from (i) the Philippines and (ii) peninsular Malaysia, Singapore and Sumatra as monophyletic lineages, separate from one another and all other sampled populations. Furthermore, our genetic analyses revealed levels of COI divergence between Wakatobi and Sulawesi populations and those from the Philippines, Sumatra, Singapore and peninsular Malaysia (see Table 3.2) that are suggestive of species-level divergence (i.e. COI divergence of 2.7% or more; Hebert et al., 2004; Kerr et al., 2007; Meier et al., 2008). This builds upon previous suggestions that the OBSB may be comprised of more than one species (see Cheke and Mann, 2008b) and that Philippine populations represent a separate species (Lohman et al., 2010). Sulawesi and Wakatobi populations showed high levels of divergence from OBSB populations west of Wallace's line and relatively low divergence from other populations on the eastern side of this line. This is not entirely surprising considering the highly complex geological history of Sulawesi and the Wallacea region and the associated high levels of avian endemism (Hall, 2002; Conservation International, 2014). Therefore, together with the findings of Lohman et al. (2010), this suggests that *C. jugularis* may contain at least three distinct sunbird species; one from the Philippines (including *C. j. jugularis* and *C. j. obscurior*, another including all other populations west of Wallace's line and another comprised of all populations to the east of Wallace's line.

While genetic distances are a good indicator of species-level differences (Hebert et al., 2004; Meier et al., 2008), they cannot be used independently for species delimitation (Winker, 2009). Future work to unravel the complex relationships within *C. jugularis* and accurately identify distinct species from this group would ideally combine genetic and phylogenetic analyses with comparisons of phenotypic traits such as plumage, morphology, acoustics, behaviour and ecology from a diversity of populations across the species' current range. The identification of evolutionarily distinct species may have major conservation implications considering the many threats facing the flora and fauna of Southeast Asia (Sodhi et al., 2004).

Our estimates of genetic divergence and inferred phylogenetic relationships suggest that Sulawesi populations are more closely related to those from the Solomon Islands

and Australia (thousands of kilometres away) than to Wakatobi populations (just 27 km away). This pattern is surprising, suggesting that selection pressures experienced by Wakatobi populations are stronger than the pressures on Sulawesi, the Solomon Islands and Australia. Additionally, this suggests the colonisation of the Solomon Islands and Australia by OBSBs (most likely from Asia; see Cheke and Mann, 2008b) has only occurred relatively recently, with insufficient time for genetic differences to accumulate. If we were to adopt the (probably overs-simplified) "rule" of 2% divergence per million years in avian, protein-coding mitochondrial DNA (Lovette, 2004), it would suggest that Solomon Island populations have been diverging from those on Sulawesi for only 150,000 years, while Wakatobi populations have been diverging from one another for at least 650,000 years and up to 1 million years (see Table 3.1). More research is required to further elucidate this pattern of divergence.

Considering the results from our genetic, phylogenetic and morphological analyses, as well as known plumage differences between Wakatobi and Sulawesi populations of *C. jugularis*, we support the current classification of Wakatobi populations as a subspecies, *C. j. infrenatus*, a recently diverged, incipient species. The Wakatobi populations, as an incipient species, provide an opportunity to study an intermediate stage in the process of speciation and identify the primary drivers of divergence in this island population. Furthermore, we draw attention to the need to resolve species limits within *C. jugularis*.

chapter 4

Competitively-mediated micro-evolution: ecological character displacement in a pair of island-dwelling birds

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Author contributions: SBAK, DJK and NMM conceived and designed the project. SBAK, DJK, AB, KA and NMM performed the field and lab work. SBAK analysed the data. SBAK wrote the manuscript with input from DJK and NMM.

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4.1 Abstract

Competitive interactions among organisms play a major role in the processes of speciation and adaptive radiation. The adaptive evolution of traits in response to interspecific competition for resources is known as ecological character displacement (ECD). ECD facilitates species co-existence by minimizing levels of interspecific competition via resource partitioning. We tested the hypothesis that *Zosterops chloris* from the Wakatobi archipelago, South-east Sulawesi, Indonesia, had undergone ECD in sympatry with a congeneric competitor. Our combined behavioural, morphological and isotopic results support this hypothesis as, in sympatry, *Z. chloris*: (i) competed both directly and indirectly for food resources with its congener; (ii) had a significantly shorter bill compared to neighbouring allopatric populations; and (iii) appears to have partitioned arthropod prey resources with its congener as a result of competition. We believe this is the first case of ECD described from the Zosteropidae and discuss the potential for future research within this remarkable family. Furthermore, we demonstrate the novel use and potential application of stable isotope analysis to this field of research. As species' ranges shift in response to climate change, creating novel competitive environments, an increased understanding of ECD will be essential for the prediction of future trends in biodiversity.

4.2 Introduction

Competitive interactions among organisms are increasingly believed to play a major role in the generation, maintenance and loss of biodiversity (Pfennig and Pfennig, 2012b). These interactions are understood to be at their most intense between closely related and ecologically similar species (MacArthur, 1976). If the costs of competition are sufficiently high, the inferior competitor may be driven to local extinction; this is known as competitive exclusion and demonstrates the power of such interactions (Gause, 1934; Hardin, 1960). Alternatively, competitively-mediated selection may lead to the adaptive evolution of traits in the interacting species that minimize competition for resources and facilitate stable co-existence. This process is known as ecological character displacement (ECD) (Brown and Wilson, 1956) and has significant theoretical (Slatkin, 1980; Taper and Case, 1985; Lande, 2009) and empirical support from a diverse range of taxa (reviewed in: Schluter, 2000a; Dayan and Simberloff, 2005; Stuart and Losos, 2013). As ECD produces an adaptive evolutionary response that facilitates species co-existence, it is thought to be of great importance in the structuring of biological communities (Dayan and Simberloff, 2005) and the processes of speciation and adaptive radiation (Schluter, 2000a; Grant and Grant, 2008).

ECD is typically demonstrated as a greater difference in resource-exploiting traits, such as bill or jaw size, between two species in sympatry compared to where they occur in allopatry. However, in order to identify interspecific resource competition as the selective agent driving evolutionary change, one must rule out alternative processes such as species sorting (i.e. only species sufficiently different upon initial encounter can co-exist) which may produce similar patterns (Pfennig and Pfennig, 2012b). By demonstrating a high degree of environmental similarity between sites of sympatry and allopatry one can rule out environmental stimuli as drivers of evolutionary divergence. Comparisons of tarsus length and body size in birds, for example, can be used to infer the level of similarity in foraging environment and resource availability between sympatric and allopatric sites (Miles and Ricklefs, 1984; Scott et al., 2003). To determine that the traits in question have indeed undergone evolutionary shifts as a result of competition for resources, one must demonstrate that changes in trait values are associated with divergence in resource use. Thus, as competitive interactions among sympatric species may lead to divergences in resource use, resource partitioning is an essential component of ECD. Competing species may partition resources among themselves spatially, occupying

different habitats or microhabitats; temporally, utilizing resources at different times; or phenotypically, monopolizing or acquiring different parts of the same resources, e.g. partitioning according to prey type or size (see Schoener, 1974). A classic example of this was documented in Darwin's (Galapagos) finches, where competitively-mediated selection led to an evolutionary divergence in bill size and associated prey size (Grant and Grant, 2006). Grant and Grant's study demonstrated that evolutionary change can occur over an ecological time-scale, becoming a landmark study in eco-evolutionary dynamics. Furthermore, to describe a robust case of ECD, a study should provide evidence to suggest that the observed pattern has not occurred by chance; that sympatric species actively compete; that phenotypic differences are a result of actual evolutionary shifts; and that such differences have a genetic basis (see Schluter and McPhail, 1992).

As resource competition is central to the theory of ECD, knowledge of the trophic ecology and habitat use of interacting species is essential to any study. Stable isotope analysis has recently become widely recognised for its applicability in ecological studies (West et al., 2006; Inger and Bearhop, 2008) and is commonly used to infer the trophic and environmental ecology, as well as isotopic niche width, of individuals, populations and species (Newsome et al., 2007; Jackson et al., 2011). The ratio of ^{15}N to ^{14}N (calculated relative to an international standard and expressed as $\delta^{15}\text{N}$) in animal tissues, such as feathers, increases in a stepwise manner by approximately 2-5‰ with trophic position and also allows one to make inferences about diet (DeNiro and Epstein 1981; Kelly et al. 2012). The ratio of ^{13}C to ^{12}C (calculated relative to an international standard and expressed as $\delta^{13}\text{C}$) increases at approximately 1‰ relative to diet and reveals the primary carbon sources in a food web, allowing inferences to be made regarding foraging habitat (DeNiro and Epstein, 1978; Kelly, 2000). While interspecific competition has been shown to drive changes in diet and habitat use (e.g. Schluter and McPhail, 1992), ecological theory also suggests that a species' niche changes with the level of interspecific competition experienced (Van Valen, 1965; Bolnick et al., 2010). Thus, stable isotope analysis offers an efficient method by which to investigate the effects of interspecific resource competition on isotopic niche width, trophic position, habitat use and diet. While a few studies have used stable isotope analysis to investigate relationships between functional morphology and resource use (e.g. Wolf et al., 2008; Bolnick and Paull, 2009; Navarro et al., 2009; Bolnick and Araújo, 2011), to our knowledge, none have used this method in the study of ECD.

Studies of island birds have been central to the development of the theory of ECD since its inception (Brown and Wilson, 1956) and today provide some of the best studied and well-known natural examples (see Dayan and Simberloff, 2005). Studies of island flora and fauna have many advantages in the study of ECD, typically having simpler communities that are easier to characterize in comparison to continental mainland. Two white-eye species (Aves: Zosteropidae) occur on the Wakatobi archipelago, South-east Sulawesi, Indonesia (see Figure 4.1). One species, the lemon-bellied white-eye (LBWE) *Zosterops chloris* is widespread across the archipelago, while the other *Zosterops sp.* is confined entirely to the northern-most island of Wangi-wangi (hence, this undescribed species has been dubbed the 'Wangi-wangi white-eye'; WWWE hereafter) (Kelly and Marples, 2010). Thus, the two species only occur in sympatry on the island of Wangi-wangi. Considering the generalist diet and typically conserved morphology of *Zosterops* species (van Balen, 2008), as well as the reduced resource diversity of small islands (MacArthur and Wilson, 1967), these species likely experienced high levels of interspecific resource competition upon initial contact on Wangi-wangi. We hypothesised that the LBWE on Wangi-wangi have undergone ECD as a result of competition with WWWE, thus facilitating co-existence. We investigated this hypothesis by studying the behavioural ecology, morphology and isotopic ecology of both species in sympatry and allopatry. We compared populations of LBWE on Wangi-wangi to those on the island of Oroho, less than 1 km offshore, where WWWE were not present. As no other WWWE populations are currently known to exist (Kelly and Marples, 2010), no such comparisons could be made for WWWE. Our results support our hypothesis, suggesting that LBWE on Wangi-wangi have undergone ECD. We believe this is the first documented case of ECD in the Zosteropidae family and demonstrate the novel use and potential applications of stable isotope analysis to this field of research.



Figure 4.1: Plumage comparison of 'Wangi-wangi' white-eye (left) and lemon-bellied white-eye (right) from the Wakatobi archipelago. Photo credit: DJK and NMM.

4.3 Methods

4.3.1 *Study site*

The Wakatobi Islands lie approximately 27 km due east of Buton Island, off the south-eastern peninsular arm of Sulawesi, Indonesia. The uplifted coral which forms the basal geology of the Wakatobi islands, which are of Australian-New Guinea origin (Hall, 2002), is said to be no more than five million years old (see Kelly and Marples, 2010). Our study compared WWWE and LBWE populations on the islands of Wangi-wangi and Oroho (also known as Kampenaune) as part of a larger study on the geographical variation of South-east Sulawesi's avifauna. All behavioural observations and mist-netting were carried out in habitats primarily composed of farmland or scrubland, where both species were known to thrive (pers. obs.).

4.3.2 *Behavioural observations and plant sampling*

Behavioural observations were completed over one week periods in August 2012 and July 2013. Line transects measuring 1 km in length were established in the previously mentioned habitats and walked at a recommended pace of 1-2 km/hr (Bibby et al., 2000). On each transect, we recorded the habitat use, density, interspecific interactions, feeding and flocking behaviour of each *Zosterops* species. If the ratio of birds to observers was 1:1 when a flock or group of individual birds was encountered during a transect, a focal

sampling method was employed; when there were more birds than observers a scan sampling method was used, recording behaviours every 10-15 seconds (for details on methods see Martin and Bateson, 1993).

In order to generate baseline isotopic data and make standardized comparisons of isotopic data across islands (Post, 2002), samples from three commonly occurring plant species were taken from transect sites on Wangi-wangi and Oroho in 2012. A cutting of approximately 2 cm² was taken from the tips of coconut (*Cocos nucifera*) leaves at 1.5-2 m in height. Four to five flower heads were taken from devil weed (*Chromolaena odorata*) plants and three to five ripe fruits were taken from lantana (*Lantana camara*) plants; all sampled at a height of approximately 1 m. All samples were stored in individual tubes of 90% ethanol, out of direct sunlight.

4.3.3 *Morphological and feather sampling*

Birds were mist-netted opportunistically on Wangi-wangi and Oroho during the months of July, August and September in 2003, 2005, 2010 and 2012. Mist nets were erected before dawn and checked in constant rotation, with processing time kept to a minimum (less than five minutes) to avoid heat-stress in the birds. Several morphological measurements were taken from each bird caught, as well as details on age, sex and breeding condition. The morphometrics taken (by NMM only) included wing length (maximum chord), weight, bill length (to skull), total head length (head plus bill) and "minimum" tarsus length (Redfern and Clark, 2001). Finally, approximately 5-10 contour feathers were plucked from the flank of each bird and stored in sealed paper envelopes. This method of feather sampling minimised the risk of injury to the birds and avoided disruption to flight ability and plumage-based visual signals (McDonald and Griffith, 2011).

4.3.4 *Morphometric analysis*

Comparisons were drawn between Wangi-wangi and Oroho populations of LBWE, as well as between WWWE and LBWE from Wangi-wangi, for wing length, weight, tarsus length, bill and skull (total head minus bill) length. Sequential Holm-Bonferroni corrections (Holm, 1979) were applied to all resulting p-values in order to correct for multiple comparisons. Effect sizes (ES) statistics were used to estimate the magnitude of differences between populations for each morphological character, using Hedge's unbiased d (see Nakagawa and Cuthill, 2007).

4.3.5 *Stable isotope analysis*

Prior to analysis, both feather and plant samples were cleaned and dried. Feather samples were cleaned using a sterile brush and 90% ethanol in order to remove potential contaminants such as preen oils. Samples were then air-dried at room temperature in sealed envelopes. Sections of barbs were cut from the distal end of the feather vane to attain a sample weight of 0.3-0.6 mg. Plant samples were oven-dried in individual trays for 24 hours at 60°C and subsequently ground into a homogenous mixture using a sterile pestle and mortar. Due to significant variation in the compositional proportions of carbon and nitrogen in plants, each sample had to be run for carbon and nitrogen separately. In order to attain an accurate reading of nitrogen values from plant samples, an ascarite trap was added to absorb carbon dioxide. Sample weights of approximately 0.5 mg and 7-8 mg were used for carbon and nitrogen analyses, respectively. Feather and plant samples were placed in individual tin capsules which were loaded and run in a Thermo DeltaPLUS continuous flow isotope ratio mass spectrometer (CFIRMS) at Trinity College Dublin. The resulting stable isotope ratios were compared to international reference standards. The reference standards used for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were AIR (atmospheric nitrogen) and VPDB (Vienna Pee Dee belemnite), respectively. Samples of an intra-lab L-alanine standard were regularly run between feather samples in order to determine the standard deviation for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ readings. Final stable isotope ratios are reported as parts per thousand (‰).

Isotopic data generated from feathers were standardized against their respective island's primary production levels (plant samples). Multivariate ANOVA models, incorporating $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data, were used to draw comparisons of trophic ecology between populations. Post hoc tests were used to draw comparisons between populations for just $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Sequential Holm-Bonferroni corrections were applied to all p-values to correct for multiple comparisons (Holm, 1979). We used Bayesian analyses to quantify and compare isotopic niche width between populations (Jackson et al., 2011). Using the SIAR package, which employs a Bayesian framework to take account of any uncertainty in isotopic data and incorporates error arising from the sampling process (Parnell et al., 2008, 2010), we generated a posterior distribution of 10^5 estimates of the standard ellipse area for each population. We then calculated the posterior probability of whether two populations differed in their isotopic niche width. All statistical analyses were carried out in R v.2.15.0 (R Development Core Team, 2013).

Table 4.1: Morphometric comparisons between LBWE from Wangi-wangi and (A) LBWE from Oroho, and (B) WWWE. The mean and standard error for each morphological character is shown, as well as the resulting corrected p-value and effect size (ES) for each comparison.

Trait	Wangi-wangi	(A)		(B)	
		Oroho	p-value; ES	WWWE	p-value; ES
Bill length (mm)	13.1 ±0.1	13.6 ±0.1	0.042 ; 0.867	17.2 ±0.1	<0.001; 6.091
Wing length (mm)	53.2 ±0.2	53.1 ±0.3	0.651; 0.181	62.9 ±0.2	<0.001; 6.741
Weight (g)	9.3 ±0.1	9.1 ±0.1	0.325; 0.444	14.4 ±0.1	<0.001; 7.501
Tarsus length (mm)	16.5 ±0.1	16.2 ±0.1	0.283; 0.515	19.3 ±0.1	<0.001; 5.071
Skull length (mm)	14.9 ±0.1	14.6 ±0.1	0.175; 0.69	16 ±0.1	<0.001; 1.729

4.4 Results

On Wangi-wangi, where the species co-occur, approximately 68% of LBWE and 66% of WWWE feeding observations were of arthropod predation from vegetation surfaces. The remainder of LBWE observations comprised nectar (29%) and fruit (3%) feeding, while the rest of WWWE observations comprised fruit feeding (34%). LBWE and WWWE were seen to interact agonistically on two of the seven occasions they were observed together. On both of these occasions an individual WWWE aggressively displaced a LBWE (one of which was perched in a fruiting bush). Furthermore, on Wangi-wangi both species were seen to occupy the same habitats, mixed farmland and scrubland, and were seen flocking together on five separate occasions (in which no agonistic interspecific interactions were recorded). LBWE occurred at densities (296 birds per km²) an order of magnitude higher than WWWE (21 birds per km²) within the habitats surveyed. Severe weather conditions made the collection of detailed behaviour data on Oroho impossible. However, LBWE were recorded in the same habitats on Wangi-wangi and Oroho during fieldwork.

Morphometric analyses of LBWE from Wangi-wangi (N = 75) and Oroho (N = 9) revealed that on Wangi-wangi, in the presence of a congeneric competitor, LBWE had a significantly shorter bill (see Table 4.1). In comparison, these two LBWE populations did not differ significantly with respect to wing length, weight, tarsus length or skull length (see Table 4.1). WWWE (N = 41) were significantly larger than LBWE on Wangi-wangi for all morphological characters measured (Table 4.1).

Results from our initial isotopic comparisons suggested that the LBWE of Oroho (N = nine birds) and LBWE of Wangi-wangi (N = 31 birds) differed significantly in their trophic ecology (MANOVA; Wilks = 0.172, F_{1,38} = 89.017, p < 0.001; Figure 4.2). However, post hoc

tests revealed that this difference was only true for carbon sources ($\delta^{13}\text{C}$; $t = -13.833$, $df = 17.894$, $p < 0.001$) and that the two populations occupied a similar trophic position ($\delta^{15}\text{N}$; $t = -1.722$, $df = 25.053$, $p = 0.097$). The observed difference in carbon sources is likely due to an increased marine influence, and thus increased carbon signature (Peterson and Fry, 1987), on the smaller island of Oroho. Our Bayesian analyses determined that Wangi-wangi and Oroho populations of LBWE had an isotopic niche of similar width (66% posterior probability of difference). The co-existing LBWE and WWWE (N = 24 birds) of Wangi-wangi were very similar in their trophic ecology (MANOVA; Wilks = 0.912, $F_{1,53} = 2.509$, $p = 0.182$; Figure 4.2). Post hoc tests confirmed there were no significant differences in carbon sources ($\delta^{13}\text{C}$; $t = 1.684$, $df = 52.975$, $p = 0.182$) or trophic position ($\delta^{15}\text{N}$; $t = 0.949$, $df = 47.898$, $p = 0.347$). Furthermore, our Bayesian analyses suggested WWWE and LBWE of Wangi-wangi had isotopic niches of similar width (55% posterior probability of difference).

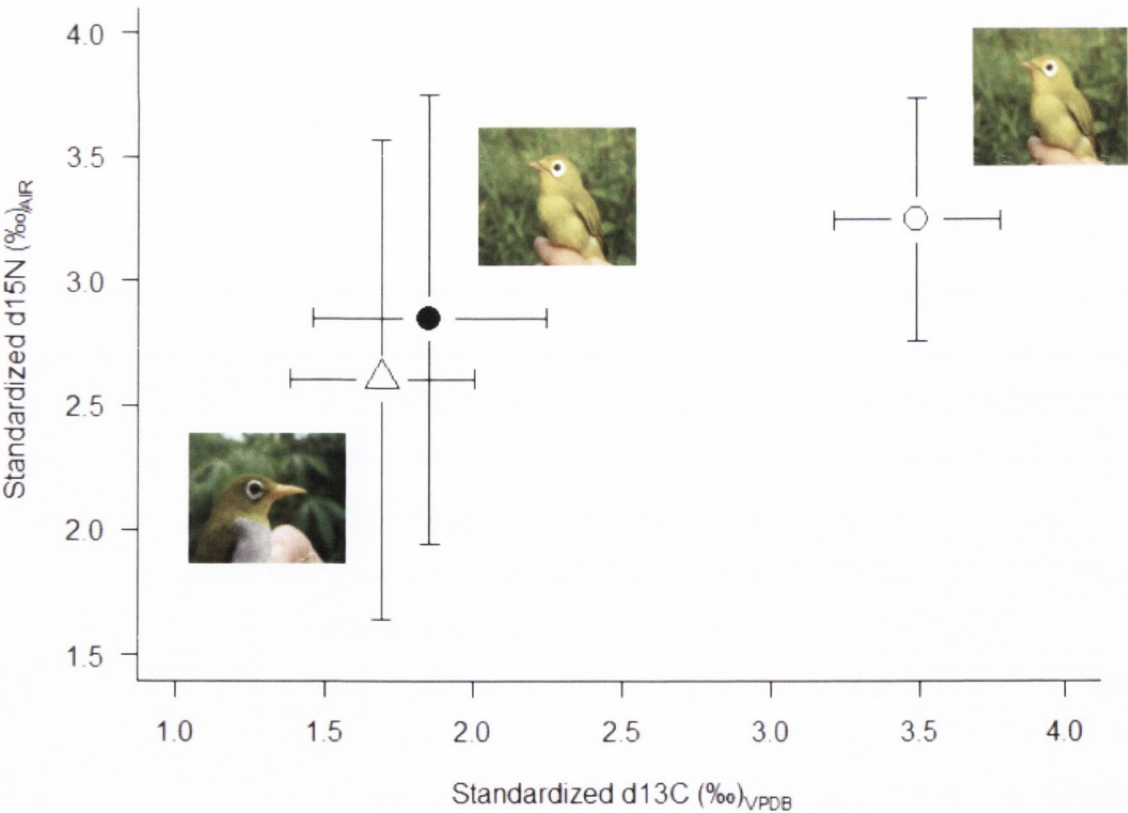


Figure 4.2: Trophic ecology of WWWE (open triangle), LBWE from Wangi-wangi (solid circle) and LBWE from Oroho (open circle). Points show the mean value $\pm$ standard deviation of the standardized $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for each population.

4.5 Discussion

Our results strongly support the hypothesis that the LBWE of Wangi-wangi Island have undergone ECD as a result of competition for food resources with WWWE. The LBWE of Wangi-wangi had a significantly shorter bill in comparison to an allopatric population on Oroho, where WWWE were absent. Indeed, the LBWE of Wangi-wangi had a significantly shorter bill compared to LBWE on all other Wakatobi islands, while the LBWE of Oroho had a bill consistently similar in length to all Wakatobi populations, other than those on Wangi-wangi (see Supplementary Material c.1). Although our isotopic data on trophic position and dietary range suggest there were no differences in resource use between allopatric LBWE populations, isotopic and behavioural data from sympatric LBWE and WWWE indicate high potential for interspecific competition. LBWE and WWWE were seen to occupy the same habitats and even flock together on Wangi-wangi. Our isotopic and behavioural data suggest they occupied similar niche space and consumed a similar range of food resources. Furthermore, we observed these populations competing directly and indirectly for arthropod prey, which comprised roughly two-thirds of each population's diet. We therefore propose that competitively-mediated selection has led to the partitioning of food resources between sympatric LBWE and WWWE. As changes in a passerine bird's bill morphology - its primary resource acquisition trait - typically reflect differences in resource use (Grant, 1968; Clegg and Owens, 2002; Grant and Grant, 2006), the decrease in LBWE bill length on Wangi-wangi is likely to be an adaptation for the consumption of smaller arthropod prey. Partitioning arthropod resources according to size is likely to have mediated and reduced interspecific competition, thus facilitating co-existence of LBWE and WWWE on Wangi-wangi.

The partitioning of prey by size among individuals and populations has been shown to correlate strongly with the size of resource-exploiting morphological traits, such as bill and jaw size, across a variety of taxonomic groups (Schoener, 1974; Gladfelter and Johnson, 1983; Aldridge and Rautenbach, 1987; Adams and Rohlf, 2000; Grant and Grant, 2006). It is therefore likely that the larger-bodied and larger-billed WWWE preyed upon larger arthropods in comparison to the smaller LBWE of Wangi-wangi (see Table 4.1). We believe that significant overlap in habitat use and diet between these populations would have led to initially high levels of interspecific competition. Competitively-mediated selection would have favoured those individuals who overlapped least in their resource-use and led to phenotypic divergence (Losos, 2000). Selection, whether as a result of interspecific

competition or environmental variation, has been shown to produce rapid and significant changes in bill morphology over just a few generations (Grant and Grant, 1995, 2006). Clegg et al. (2008) showed that even weak directional selection can produce substantial morphological divergence in island white-eyes. Furthermore, it has been shown that some island-dwelling white-eyes which are seemingly generalist foragers actually comprise a range of individual specialists (Scott et al., 2003). Competitively-mediated selection may act upon such standing variation in the ecology and morphology of a population and initially facilitate ECD, potentially resulting in an adaptive evolutionary change in one or both species and thus the avoidance of competitive exclusion (Pfennig and Pfennig, 2012a).

Our behavioural, morphological and isotopic data provide evidence to wholly or partially satisfy five of the six criteria of ECD outlined by Schluter and McPhail (1992) (see Appendices, Tables c.1 and c.2). While these criteria prove very useful in defining and positively identifying cases of ECD, it has been acknowledged that trying to satisfy all six criteria is a difficult feat and could even result in overlooking true cases of ECD (Pfennig and Pfennig, 2012b; Stuart and Losos, 2013). With no allopatric WWWE populations to draw comparisons with, we cannot determine whether WWWE have diverged phenotypically as a result of competition with LBWE on Wangi-wangi.

Previous research has shown that passerine bill morphology is highly heritable (Alatalo and Gustafsson, 1988; Grant and Grant, 1994, 1995; Keller et al., 2001). While changes in a resource-exploiting trait such as a bird's bill may initially be produced as a plastic response to an environmental selection pressure, phenotypic changes can be genetically assimilated over a few generations and become constitutively expressed, even after the original environmental pressure is removed (Waddington, 1953; Aubret and Shine, 2009; Lande, 2009; Moczek et al., 2011). In Darwin's finches, bill size and shape are regulated by single gene pathways (Abzhanov et al., 2004, 2006). It is therefore likely that bill morphology is very susceptible to micro-evolutionary changes and that differences in LBWE bill morphology have a genetic basis. Changes in bill size among populations of the same species have been linked to divergence in song (Podos, 2001; Podos and Nowicki, 2004; Barbara et al., 2013). It has also been shown that a population's song may change in the presence of a new heterospecific competitor (Grant and Grant, 2010). The modification of a key mate-signalling process such as a bird's song could potentially promote reproductive divergence between populations. Therefore, adaptive phenotypic changes driven by competition for resources, such as the observed divergence in LBWE

bill size, could ultimately lead to reproductive isolation between allopatric populations and thus speciation.

While our findings demonstrate that the LBWE had a shorter bill in the presence of a congeneric competitor, our isotopic results indicated no significant variation in niche width between LBWE populations. Theory predicts that in the presence of increased interspecific competition, LBWE of Wangi-wangi would have occupied a narrower niche compared to Oroho populations (van Balen, 2008; Bolnick et al., 2010). A recent study suggests that we may have expected to see a corresponding change in isotopic niche width with changes in bill size (Hsu et al., 2014). However, while our isotopic results would reveal changes in the diversity of food resources consumed, they are unlikely to reflect the partitioning of resources of the same class according to type or size. We recommend future studies run isotopic analyses of known food items and employ Bayesian mixing models (Parnell et al., 2013) to more accurately determine diet. While stable isotope analysis is not likely to deem more traditional methods such as stomach content analysis redundant, it does offer a more exact and efficient method by which to estimate diet, trophic ecology and niche width. We believe this to be the first study to employ stable isotope analyses in the study of ECD and encourage its use in future research.

To our knowledge, this is to the first case of ECD described in white-eyes (*Zosteropidae*), a remarkable family of birds renowned for their dispersal and colonisation abilities, having colonised more islands globally than any other passerine group (Moreau, 1964; van Balen, 2008). White-eyes have one of the highest diversification rates among vertebrates (Moyle et al., 2009) and have produced adaptive radiations that rival even those of Darwin's finches for tempo and diversity (Melo et al., 2011). Furthermore, species from the *Zosterops* genus are found in sympatry with congeners more commonly on islands than on mainland and multiple colonizations of the same island are well known (van Balen, 2008). Numerous studies have provided great insight into the ecology and evolution of island white-eyes, as well as the processes of population divergence and speciation in general (Clegg et al., 2002a,b, 2008; Milá et al., 2010; Melo et al., 2011). Yet, despite this, there have been no studies of ECD in this group of birds. White-eyes are commonly kept as pets (Nash, 1993) and their suitability as laboratory subjects has been demonstrated (Wiltschko et al., 1998; Potvin and Mulder, 2013). We therefore suggest that white-eyes would be an excellent model group for future laboratory- and field-based research into competitively-mediated

selection, the mechanisms of ECD and the traits that facilitate it.

Our findings suggest the LBWE of Wangi-wangi Island have undergone micro-evolutionary change as a result of interspecific competition for resources, supporting the theory of ECD and demonstrating the powerful role competition may play in driving and maintaining divergence among organisms. Furthermore, we have demonstrated the potential application of stable isotope analysis to studies of ECD and believe this to be the first case of ECD described from the white-eye family (Zosteropidae), a group with great potential for future laboratory- and field-based research. Our study provides further evidence that novel competitive interactions among species can produce adaptive evolutionary responses which reduce competition and facilitate co-existence, typically resulting in an evolutionary divergence in one or both species. Such evolutionary change may play a significant role in the processes of speciation and adaptive radiation (Schluter, 2000b; Pfennig and Pfennig, 2012a; Price et al., 2014).

chapter 5

Competition for food resources drives body size divergence in island birds

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Author contributions: SBAK, DJK and NMM conceived and designed the project, and carried out the field work. SBAK analysed the data. SBAK wrote the manuscript with input from DJK and NMM.

Status: This manuscript is being preparation for publication (with the consideration of consolidating chapters 4 and 5 into a single manuscript). Potential target journals include the Journal of Animal Ecology (and the Proceedings of the Royal Society B for a consolidated paper).

5.1 Abstract

An understanding of the processes that drive phenotypic diversification is central to our understanding of speciation. Island birds show repeated patterns of divergence in various phenotypic traits relative to mainland populations. One of the most pronounced changes occurs in body size, with passerine birds typically becoming larger on islands. Selection as a result of competition, predation, parasitism and other processes has been suggested to drive these patterns. Combining data on the behavioural ecology of two passerine species, lemon-bellied white-eye (*Zosterops chloris*) and olive-backed sunbirds (*Cinnyris jugularis*), the density of available food resources and the biomass of potential competitor species, we investigated whether variation in the levels of inter- and intra-specific competition explained variation in body size across an island chain. Our results described species-specific patterns. The body size of *Z. chloris* increased as the availability of food resources decreased, supporting the dominance hypothesis: that island passerines evolve larger body sizes as a result of selection for success in agonistic interactions. Conversely, *C. jugularis* exhibited reduced body size when the number of potentially competing species increased by one. This result suggests interspecific competition is a major driver of body size divergence in *C. jugularis* and provides some support for the niche variation hypothesis. Together, our results provide further evidence of the powerful role of competition in phenotypic evolution and suggest the specific mechanisms driving divergence will vary depending on the species.

5.2 Introduction

Phenotypic variation among living organisms has always fascinated and enthralled humanity. Undoubtedly, studies of phenotypic variation have led to the development of some of the most influential theories in the biological sciences. For example, the striking intergradation of phenotypic forms across geographic regions was a great source of wonder and inspiration to Darwin and Wallace (1858) in their development of the theory of evolution by natural selection. Indeed, it was interest in the diversity of phenotypic traits in the pea plant (*Pisum sativum*) that led Gregor Mendel (1866) to his hypothesis on genetic inheritance, the foundation of modern genetics. Our knowledge of the patterns of phenotypic variation and the means of trait inheritance has increased markedly since these seminal works (e.g. Abzhanov et al., 2006; Meiri et al., 2011), but we have only recently acquired a more comprehensive understanding of the evolutionary processes that generate and maintain phenotypic variation (e.g. Price, 2008; Pfennig and Pfennig, 2012a).

Body size is one of the most apparent phenotypic traits in vertebrate animals, exhibiting remarkable variation in the present day, ranging from the 170 ton blue whale (*Balaenoptera musculus*) that reaches 30 m in length (Deméré, 2014), to a Madagascan dwarf chameleon (*Brookesia micra*) that weighs just 0.2 g and reaches less than 30 mm in length (Glaw et al., 2012). In comparison to mainland areas, the many physical, environmental and biological properties of islands are understood to lend themselves to the evolution of extreme forms that exhibit either gigantism or dwarfism (Whittaker and Fernández-Palacios, 2007). Thus island fauna have, historically, received much attention from both the public and scientific community. Among these, island birds have been well studied due to their remarkable diversity of body sizes, from the infamous dodo (*Raphus cucullatus*) and the gigantic, terrestrial moa (*Dinornis*) birds from New Zealand (Worthy and Holdaway, 2002) to the tiny bee hummingbird (*Mellisuga helenae*) of Cuba which reaches just 6 cm in length and 2.6 g in weight (Chai and Kirwan, 2014).

Numerous hypotheses have been proposed to explain global patterns of body size variation in birds and other vertebrates. One hypothesis, known as Bergmann's rule (Bergmann, 1847), which has much support from macroecological analyses (Ashton, 2002; Meiri and Dayan, 2003), suggests that the body size of birds and mammals increases with latitude as an adaptation to colder climates. This co-variation in body size and

temperature is likely to be an adaptation for the optimisation of an animal's metabolism and physiology relative to local environmental temperatures (Hamilton, 1961; Grant, 1968; Blondel, 2000). Another hypothesis, the 'island rule', attempts to explain the evolution of insular gigantism and dwarfism, suggesting that insular forms converge on a medium body size, with large forms becoming smaller and small forms becoming larger (Foster, 1964). While some studies have provided evidence in support of the island rule in birds (e.g. Clegg and Owens, 2002), more recent macroecological comparative studies found limited support for this hypothesis (Meiri et al., 2008, 2011). Alternatively, studies have described some taxon specific trends; for example, that birds typically become larger on islands (Meiri et al., 2011).

While various macro-scale patterns of body size variation have been described, it is difficult to determine the mode and strength of evolutionary processes that generated such patterns. A range of processes are hypothesised to explain body size divergence and variation in insular lineages, such as changes in: temperature (as previously mentioned Meiri and Dayan, 2003), dispersal ability (Adler and Levins, 1994), habitat complexity (Hamilton, 1961) and levels of predation (Lomolino, 1985; Beauchamp, 2010) and inter- and intra-specific competition (Grant, 1968). Differences in the competitive environment are one of the classic hypotheses proposed to explain divergence in avian body size on islands. As islands are typically species-poor in comparison to nearby continental areas of mainland (Whittaker and Fernández-Palacios, 2007), island colonists are thought to experience a competitive release as a result of decreased interspecific competition. This classic theory, known as the niche variation hypothesis (Van Valen, 1965), predicts that with competitive release, more niche space becomes available to colonists and that the evolution of a more generalist foraging behaviour and generalist morphology (which includes a larger body size) facilitates the exploitation of this newly vacant wider space (Brown and Wilson, 1956; Grant, 1968, 1972). Studies on coal tits (*Parus ater*) have shown that as the level of interspecific competition decreased, birds increased in average and maximum body size (see Dhondt, 2012). However, large body size has not been associated with generalist foraging (Werner and Sherry, 1987; Scott et al., 2003). Research suggests that it is changes in bill size, a bird's primary resource acquisition trait, and not body size, that facilitate more generalist foraging and the exploitation of wider niches (Clegg and Owens, 2002; Hsu et al., 2014).

Island birds typically occur at higher densities in comparison to their closest relatives on the mainland (MacArthur et al., 1972; Blondel et al., 1988) and it has been suggested that island forms therefore experience higher levels of intraspecific competition. The 'dominance hypothesis' suggests that if intraspecific competition for resources is high, selection will favour larger body size as it confers a competitive advantage in agonistic interactions (MacArthur, 1961; Kikkawa, 1980). In some of the first tests of the dominance hypothesis in birds, Kikkawa (1980) and Robinson-Wolrath and Owens (2003), found that in silvereyes (*Zosterops lateralis*) from Heron Island, Australia, larger body size was associated with increased dominance, fitness and survival. Clegg and Owens (2002) also showed, for numerous species, that shifts towards a larger body size were associated with higher potential for intraspecific competition. It is also thought that there is greater potential for intraspecific competition in those species that regularly feed in flocks, compared to those that do not (see Clegg and Owens, 2002).

To gain further insight into the microevolutionary processes that drive phenotypic divergence in island birds, we investigated the drivers of body size variation in two passerine species across an island chain. We tested the hypothesis that variation in the levels of inter- and/or intra-specific competition for food resources drives inter-island differences in lemon-bellied white-eye (*Zosterops chloris*) and olive-backed sunbird (*Cinnyris jugularis*) body size. Following the dominance hypothesis, and given that island environments have a limited carrying capacity relative to the quantity of available food resources (Begon et al., 2006), one would predict that when the density of available food resources is low, intraspecific competition would be high and thus birds would increase in body size in order to dominate access to resources. One would then predict that as body size increased in response to low resource availability, the density of these birds would decrease, as the environment can support a limited biomass. The niche variation hypothesis also predicts that changes in the levels of interspecific competition may drive body size variation. Therefore, combining behavioural, ecological and morphological data, this study investigated the relationship between a species' body size and: (i) the level of observed direct intraspecific competition; (ii) the level of observed direct interspecific competition; (iii) the density of the study species; (iv) the density of potential (all available) and known (confirmed via behavioural observations) food resources; and (v) the biomass of potentially competing species.

5.3 Methods

5.3.1 *Study species*

We collected behavioural, ecological and morphological data on five species from the Wakatobi Islands, South-east Sulawesi, Indonesia; two focal study species, the lemon-bellied white-eye (LBWE) and olive-backed sunbird (OBSB), and their potential competitors, the island monarch (*Monarcha cinerascens*; ISMON), Wakatobi flowerpecker (*Dicaeum kuehni*; WKFP) and 'Wangi-wangi' white-eye (*Zosterops sp.*; WWWE). These species are ecologically and behaviourally similar, occupying the same habitats, with varying overlap in the range of dietary resources consumed (i.e. terrestrial invertebrates, fruit and nectar) (Coates et al., 2008; del Hoyo et al., 2008). Thus, there is great potential for competition for food resources between these species. All of the aforementioned species are present on the four main islands of the Wakatobi archipelago (Wangi-wangi, Kaledupa, Tomia and Binongko; see Figure 1.1) except for the WWWE which is restricted to the island of Wangi-wangi (Kelly and Marples, 2010).

5.3.2 *Morphological sampling*

Sampling of LBWE, OBSB, WKFP, ISMON and WWWE took place across the four main Wakatobi islands. Birds were mist-netted opportunistically during the months of July, August and September in various years between 1999 and 2012. Mist nets were erected before dawn in areas of scrubland and mixed farmland; chosen for their accessibility and suitability. After being assessed for age (adult or juvenile) and sex, the following morphometric measurements were taken for each netted bird: wing length (maximum chord), weight, bill length (to skull), bill depth (at distal end of nares), tail length (standard), tarsus length ('minimum') and total head length (head plus bill) (Redfern and Clark, 2001). The GPS location of each netting site was recorded.

5.3.3 *Behavioural observation and resource quantification*

Data on the behavioural ecology of all study species and the density and diversity of available fruit and nectar resources were collected during two visits to the Wakatobi Islands. Binongko and Tomia were visited during April and May 2013 as transport and access to Binongko became increasingly difficult in later months. Sampling visits to Wangi-wangi, Kaledupa and Tomia were completed in July 2013. Tomia was visited on both occasions to determine whether bird and resource densities changed between sampling

periods and thus, whether data gathered on Binongko should be transformed accordingly.

Line transects measuring 1 km in length were established in the areas of mixed farmland and scrubland and walked at a recommended pace of 1-2 km/hr (Bibby et al. 2000). Where possible, transects were established in close proximity to mist-netting sites. Only birds and food resources up to 25 m either side of this transect were recorded. On each transect, the following were recorded for each bird species: density, inter- and intra-specific agonistic interactions, feeding behaviour and flocking behaviour. Density was calculated as the number of individual birds recorded within the area of the 1 km long and 50 m wide transect. Observations on feeding behaviour allowed us to investigate inter-island variation in diet but also determine the specific food resources consumed by each species.

While walking transects, if the ratio of birds to observers was 1:1 when a flock or group of individual birds was encountered, a focal sampling method was employed. When there were more birds than observers, the flock was split between observers and a scan sampling method employed, recording behaviours every 10-15 seconds (see Martin and Bateson, 1993). Flocks of the same size and composition thought to have been encountered earlier in the transect were not recorded. However, inadvertent recording of the same individuals/flock is not thought to be a major issue in density estimates (Bibby et al., 2000; Sutherland and Green, 2004).

Transect surveys of bird behaviour were completed between 6.00am and 8.30am during a period of peak avian activity (Bibby et al., 2000), with each transect surveyed only once. As the study species were known to feed on a variety of invertebrate, fruit and nectar resources (Coates et al., 2008; del Hoyo et al., 2008), each transect was subsequently surveyed to record the density and diversity of available fruit and nectar resources. Unfortunately, meaningful estimates of the density of available invertebrate resources demanded more time and resources than available during the expeditions. Each transect area was split between two recorders and each covered a 25 m x 1 km area, again at a pace of 1-2 km/hr, and counted the number of individual plants possessing ripe fruit or open flowers. Each plant was identified to a given morphotype if more accurate taxonomic identification was not possible.

Preliminary transects were carried out to familiarise observers with the methods, develop an ethogram of bird behaviours (to allow for more efficient data collection) and create a photographic library in which all flowering and fruiting plants were recorded and assigned a morphotype. Any other potential fruit or nectar resources and novel behaviours frequently encountered were added to the photo-library or ethogram, respectively. A GPS unit was used to measure the length and mark the locations of each transect.

5.3.4 *Data transformation and interpolation*

The most commonly used measures of body size in birds are wing length and weight (Rising and Somers, 1989). However, many avian morphological traits such as wing length and weight are highly auto-correlated (Rising and Somers, 1989; Ricklefs, 2012). Therefore, we used principal component analysis (PCA) to produce an independent variable that provided a multivariate measure of body size. In PCA of morphological data, the first or second principal component is generally an indicator of overall body size. We carried out PCA on matrices containing data on wing length, weight, tarsus length, bill length and skull length, for LBWE and OBSB separately. As our morphological variables differed in their scales, we scaled each variable prior to PCA by standardizing each measurement relative to the mean and standard deviation of that variable. As our density estimates of available and confirmed fruit and nectar resources may also be correlated (Abbas et al., 2014), we used PCA to investigate these correlations and produce independent, multivariate measures of food availability. All density estimates were scaled prior to analysis. To account for interspecific dietary variation, PCA of food resources was run separately for both LBWE and OBSB.

As we aimed to investigate how inter- and intra-specific competition drive variation in body size, we endeavoured to maximise the use of morphological data. Therefore, for each mist-netting site, and thus each bird sampled, we interpolated data on the density of each bird species, the number of inter- and intra-specific interactions and the density of potential (i.e. available) and known (i.e. confirmed via behavioural observations) nectar and fruit resources from transect data. Using the 'gstat' package (Pebesma, 2004) and the GPS data collected, we used inverse distance weighting (Shepard, 1968) to interpolate data for each mist-netting site, with the weight of each transects' data on our interpolation diminishing with distance from the site. Data interpolation was carried out for each island,

for OBSB and LBWE separately.

As behavioural and food resource data could only be collected on Binongko at a different time of year to all other islands, we collected data on Tomia during both field expeditions. We then used Poisson-distributed, generalised linear models (GLMs) to determine if the density of each bird species, density of food resources and number of inter- and intra-specific agonistic interactions on Tomia varied significantly between visits. If there were any significant differences between seasons, we multiplied all Binongko counts by the observed positive or negative change between mean counts per season on Tomia.

5.3.5 *Body size variation*

For our measure of body size, we used the principal component (PC) which was loaded predominantly by wing length and weight. Analysis of variance (ANOVA) models were used to determine whether LBWE and OBSB were sexually dimorphic in body size. We calculated effect size (ES) statistics, using Cohen's unbiased D (see Nakagawa and Cuthill, 2007), to determine the magnitude of sexual dimorphism on each island and determine whether it varied. We then used ANOVA models to determine whether body size varied significantly between islands, for OBSB and LBWE separately. As the birds sampled varied in age and sex, and more than one person measured morphological traits, we controlled for this variation in our models.

5.3.6 *Body size predictors*

We constructed linear mixed effects models (using *lmer* in the *lme4* package; Bates et al., 2014) to determine whether change in LBWE and OBSB body size were correlated with: the density of the focal species; the density of available food resources; and the biomass of potentially competing species. We controlled for the fixed effects of processor, age and sex of birds. As morphological sampling sites were nested within islands, we included sampling site as a random factor in all our models. We included all fixed and random effects in our initial models and used the likelihood ratio test (assessed using χ^2) to omit non-significant factors sequentially and determine the most suitable (i.e. 'best') model (see Zuur et al., 2009). Model selection was based on maximum likelihood and Akaike's information criterion (AIC; Akaike, 1974). Unfortunately, data on the number of inter- and intra-specific agonistic interactions were insufficient to allow for any meaningful

investigations of the relationship between these interactions and body size. All analyses were carried out in R v.3.0.2 (R Development Core Team, 2013).

5.4 Results

5.4.1 *Morphological, behavioural and resource sampling*

Across the four main islands of the Wakatobi archipelago, 461 LBWE, 110 OBSB, 20 WKFP, 93 ISMON and 48 WWWE were netted and morphological measurements taken for each bird. Forty transect surveys were completed in total; 12 on Wangi-wangi, 12 on Kaledupa, 6 on Tomia (with repetition of the same transects in May and July for comparison) and 10 on Binongko.

LBWE were recorded foraging individually or in pairs in 69% of encounters and otherwise occurred in larger flocks, ranging in size from three to 10 individuals (31% of encounters; $N = 367$ total LBWE observations). OBSB typically foraged individually or in pairs (92% of observations) and foraged less commonly in flocks of three or more individuals (8% of observations; $N = 455$ total OBSB observations).

Inter-island variation was apparent in the inferred diet of LBWE and OBSB (see Table 5.1). From observations, it was determined that WKFP diet consisted of 50% fruit, 25% invertebrates and 25% nectar, while WWWE diet was made up evenly of invertebrates and fruit, and ISMON were seen to feed solely on invertebrates. Thus, potential competitors varied in the extent of dietary overlap. In total, data on the density of 56 flowering plant species and 33 fruiting plant species were collected across our 40 food resource surveys. LBWE were recorded feeding on five different flowering plants and six different fruiting plants. OBSB were recorded feeding from 19 different flowering resources and were not recorded feeding on fruit. OBSB were also recorded feeding from the five flowering plant species used by LBWE, including *Cocos nucifera* (coconut), *Lantana camara* (red sage) and *Manihot esculenta* (cassava). LBWE and OBSB were seen to interact agonistically three times, in each of which LBWE were displaced by OBSB. While OBSB ($N = 4$) were also seen to be dominant over WKFP in agonistic interactions, LBWE were seen to be displaced by WKFP ($N = 1$) and WWWE ($N = 2$). For the ethogram of known behaviours of LBWE, OBSB, WKFP, ISMON and WWWE, see Appendices, Table d.1.

Table 5.1: The composition of LBWE and OBSB diet across the four main Wakatobi Islands. The percentage contribution of invertebrate, fruit and nectar resources is shown (based on the average number of observations per transect (*N*) on each island). Data from Tomia includes observations from July only.

	Invertebrates	Fruit	Nectar
LBWE:			
Wangi-wangi (<i>N</i> = 12)	68%	3%	29%
Kaledupa (<i>N</i> = 12)	58%	7%	35%
Tomia (<i>N</i> = 6)	65%	14%	21%
Binongko (<i>N</i> = 10)	100%	0%	0%
OBSB:			
Wangi-wangi (<i>N</i> = 12)	13%	0%	87%
Kaledupa (<i>N</i> = 12)	17%	0%	83%
Tomia (<i>N</i> = 6)	11%	0%	89%
Binongko (<i>N</i> = 10)	31%	0%	69%

5.4.2 Data transformation

Principal component analysis of LBWE and OBSB morphological data determined that wing, weight and tarsus length were all positively correlated (LBWE correlations: 0.13-0.38; OBSB correlations: 0.55-0.75), while bill length was positively correlated with these traits but to a lesser degree (LBWE: 0.12-0.28; OBSB: 0.41-0.53). For both species, skull length was weakly correlated wing length, weight and tarsus length (LBWE: 0.09-0.12; OBSB: 0.1-0.24) but, interestingly, had a relatively strong negative relationship with bill length (LBWE: -0.67; OBSB: -0.41).

The first principal component (PC1) generated from PCA of LBWE morphological data explained 36% of the variation in the data. Bill length and skull length contributed the most to PC1, while wing length, weight and tarsus length had lower loadings (PC1 loading coefficients: bill: 0.657; skull: -0.482; wing: 0.263; weight: 0.338; tarsus: 0.390). PC2 for LBWE morphology explained 30% of the variation in the data and was strongly loaded by wing length and weight (PC2 loading coefficients: wing= -0.507; weight = -0.554; tarsus = -0.278; bill = 0.255; and skull = -0.543). PC2 was chosen as the more appropriate measure of LBWE body size as it explained a similar level of variation compared to PC1 but was loaded more by wing length and weight.

PCA of OBSB morphology determined that PC1 explained 53% of variation in the data and was strongly loaded by wing length, weight, tarsus length and bill length (PC1 loading coefficients: wing = -0.54; weight = -0.54; tarsus = -0.47; bill = -0.44). PC2 explained less variation in the data (27%) and was strongly loaded by bill and skull length (PC2 loading coefficients: bill = -0.51; skull = -0.83; wt = -0.18). Therefore, PC1 was chosen as the best overall measure of OBSB body size.

PCA of the density of available and confirmed fruit and nectar resources for LBWE determined that many of these variables were correlated (with values ranging from -0.45 to 0.81). PC1 explained 56% of the variation in these data and was loaded positively by fruit density (available fruit resources: 0.442; and confirmed fruit resources: 0.613) and negatively by nectar density (available nectar resources: -0.417; and confirmed nectar resources: -0.505). PC2 explained 33% of variation in these data and was positively loaded by all resource densities (available fruit resources: 0.639; confirmed fruit resources: 0.271; available nectar resources: 0.608; and confirmed nectar resources: 0.386). As PC1 effectively provided an estimate of the ratio of fruit to nectar resources, we chose PC2 as the most suitable estimate of food availability with which to run further analyses. PCA of available and confirmed food resources for OBSB determined that PC1 explained 64% of variation in these data and was loaded by all resource densities (available fruit resources: -0.238; available nectar resources: -0.668; and confirmed nectar resources: -0.705). Note: OBSB were not recorded feeding on fruit, thus no confirmed fruit resources could be included in this analysis. Thus, PC1 was accepted as a suitable measure of the density of food available to OBSB.

We found that the densities of available nectar and fruit resources showed significant temporal variation while bird densities did not. Poisson-GLMs determined there was no significant variation in the density of LBWE ($z = 1.218$; $p = 0.223$), OBSB ($z = -1.82$; $p = 0.068$), WKFP ($z = -1.514$; $p = 0.13$) or ISMON ($z = -0.904$; $p = 0.366$) on Tomia between May and July. However, the density of total available fruit ($z = -12.71$; $p < 0.001$) and nectar ($z = -63.38$; $p < 0.001$) resources on Tomia was significantly higher in July than in May. Thus, densities of fruit and nectar resources on Binongko in May were multiplied by the observed change in mean density of the respective resource type on Tomia between May and July. This transformation was carried out for the density of available fruit and nectar resources as well as the density of known fruit and nectar resources.

Note: as no WKFP were mist-netted on Binongko there were no data on weight with which to calculate biomass estimates for the islands. Thus, the mean weight across Wangi-wangi, Kaledupa and Tomia for WKFP was used to calculate biomass estimates for Binongko. This was justified as we found no difference in the weight of WKFP when we compared populations from Wangi-wangi and Tomia ($W = 24$, $p = 0.72$), the only two islands for which we had sufficient data for such a comparison.

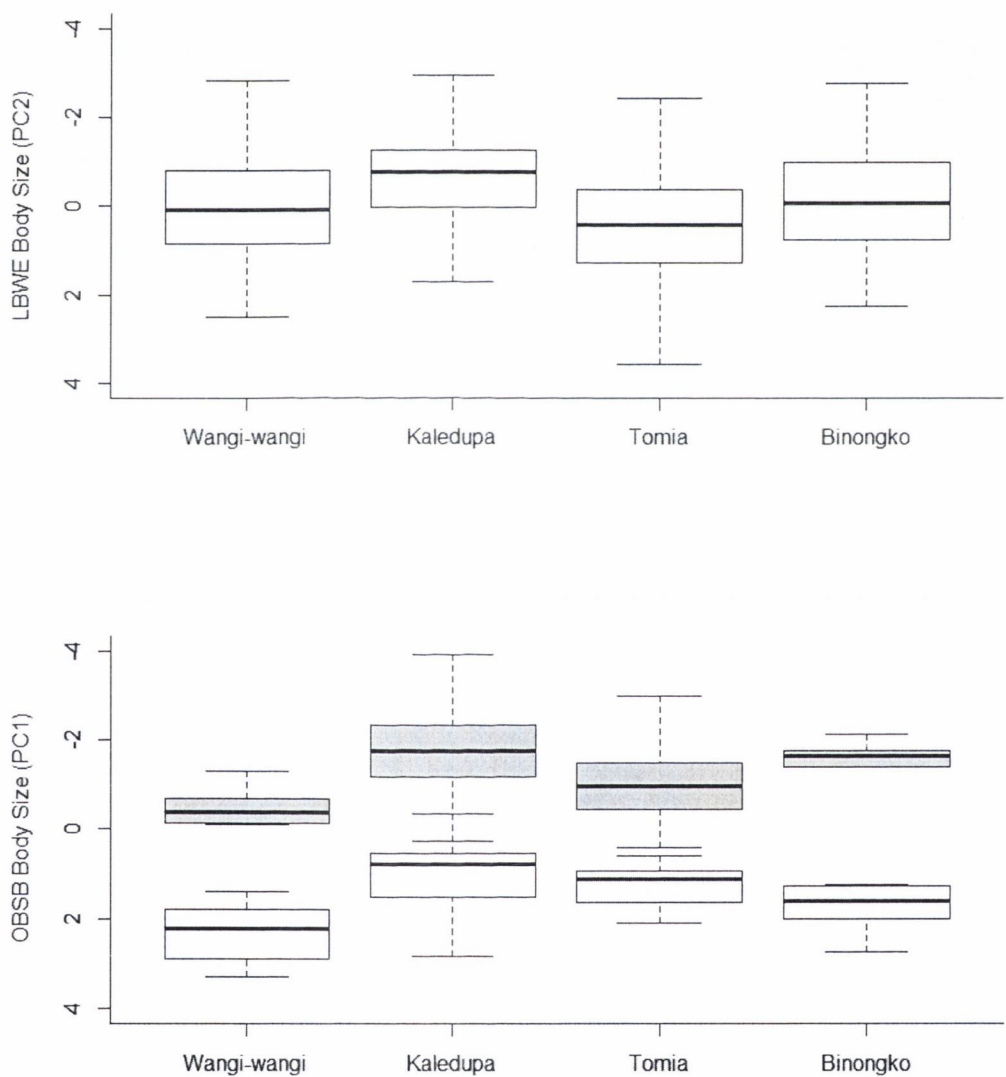


Figure 5.1: Variation in the body size of LBWE (above) and OBSB (below; with males shown in grey and females in white) across the Wakatobi Islands. In both plots, negative principal component values correspond to large values and thus y-axes have been inverted.

5.4.3 *Body size variation*

Both LBWE and OBSB displayed significant variation in body size among islands (LBWE: $F_{3,390} = 6.650$; $p < 0.001$; OBSB: $F_{3,96} = 9.997$; $p < 0.001$; see Figure 1). OBSB also exhibited high sexual dimorphism in body size ($F_{1,99} = 155.657$; $p < 0.001$). Males were larger than females on Wangi-wangi ($F_{1,18} = 76.431$; $p < 0.001$; $ES = -3.915$), Kaledupa ($F_{1,27} = 33.493$; $p < 0.001$; $ES = -2.196$), Tomia ($F_{1,28} = 49.669$; $p < 0.001$; $ES = -2.538$) and Binongko ($F_{1,17} = 66.34$; $p < 0.001$; $ES = -3.574$). The level of dimorphism in OBSB on each island was similar; with large effect sizes in each case (see Nakagawa and Cuthill, 2007). Of those birds that could be positively sexed in the field, we determined that LBWE were not sexually dimorphic for body size ($F_{2,237} = 2.712$; $p = 0.1$).

Table 5.2: Summary of linear mixed-effect model statistics for factors that predict variation in the body size of **(A)** LBWE (PC2) and **(B)** OBSB (PC1). These values are taken from the best model for LBWE and OBSB. Sampling site was included as a random factor in LBWE and OBSB models.

Factor	Estimate±s.e.	χ^2	<i>p</i>
(A)			
Intercept	0.429±0.666	-	-
Processor	0.882±0.257	11.478	<0.001
Food availability (PC2)	0.427±0.111	11.998	<0.001
OBSB Biomass	-0.017±0.005	13.133	<0.001
ISMON Biomass	0.018±0.004	14.068	<0.001
(B)			
Intercept	1.208±0.14	-	-
Age	0.983±0.353	7.47	<0.001
Sex	-2.722±0.179	120.89	<0.001
Food availability (PC1)	-0.22±0.076	7.608	<0.001
WWWE Presence	0.097±0.018	16.155	<0.001

LBWE best model: AIC = 1175.3; log-likelihood = -580.6; R-squared = 29.4%; observations (*n*) = 395; sites = 24; d.f. = 7. OBSB best model: AIC = 277.5; log-likelihood = -131.7; R-squared = 71.6%; observations (*n*) = 103; sites = 23; d.f. = 7.

5.4.4 *Body size predictors*

Our mixed effects models determined that, after controlling for variation in processor accuracy, LBWE body size (PC2) varied significantly with the density of available of food

resources, as well as the biomass of OBSB and ISMON (potential competitors); see Table 5.2(A). Our best model of these relationships explained 29.4% of variation in LBWE body size. The density of available food resources had the largest effect on LBWE body size; as the density of food increased, LBWE decreased in body size (as positive PC2 values correspond to smaller body sizes). LBWE body size decreased as ISMON biomass increased, while it increased with OBSB biomass; although, the effect of the biomass of both these potential competitors is considerably less than that of the effect of food availability.

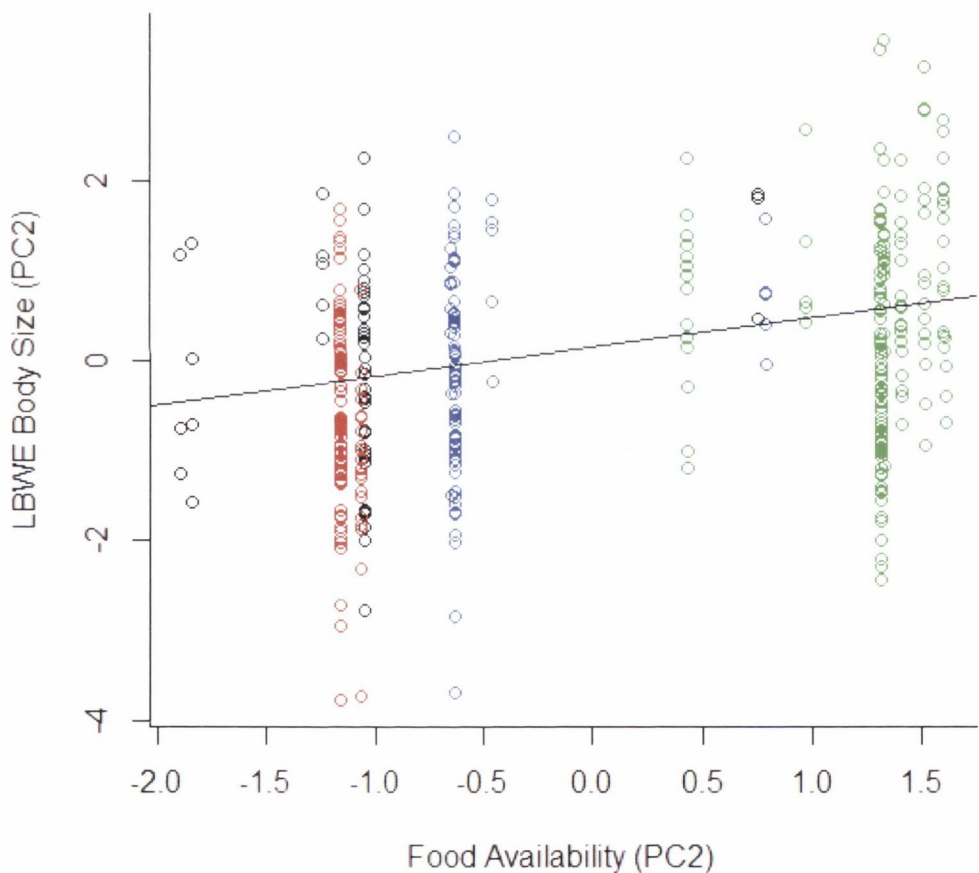


Figure 5.2: Variation in the body size of LBWE (PC2) across the Wakatobi Islands according to food availability (PC2). Islands shown: Wangi-wangi (blue), Kaledupa (red), Tomia (green) and Binongko (black). Negative principal component values for body size correspond to larger body sizes.

For OBSB, our mixed effects models determined that, after controlling for variation in the age of individual birds and the accuracy of processors, body size (PC1) varied significantly with the density of available of food resources and the presence/absence

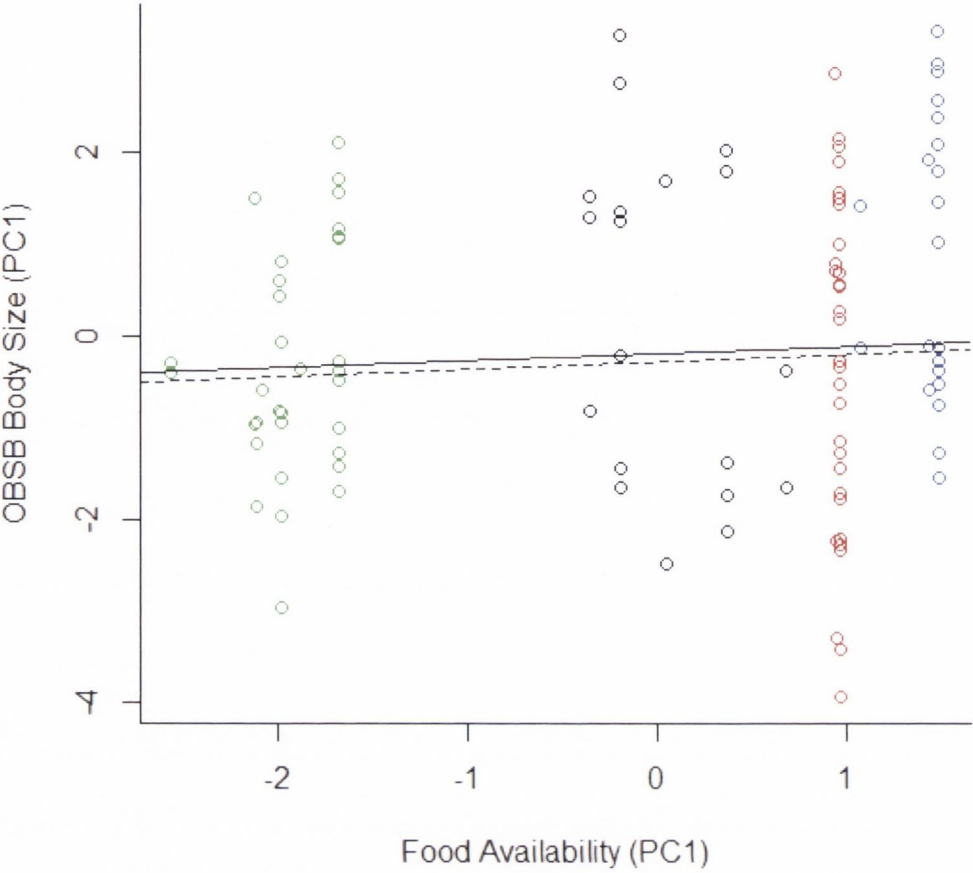


Figure 5.3: Variation in the body size of OBSB (PC1) across the Wakatobi Islands according to food availability (PC1) and the presence (solid line) and absence (dashed line) of the WWWE. Islands shown: Wangi-wangi (blue), Kaledupa (red), Tomia (green) and Binongko (black). Negative principal component values for body size and food availability correspond to larger bodies and higher food availability.

of WWWE (see Table 5.2(B)). Our best model of these relationships explained 71.6% of the variation in OBSB body size. As the density of available food resources increased, OBSB body size increased (see Figure 5.3). On Wangi-wangi Island, where WWWE were present, OBSB were significantly smaller (see Figure 5.3). Note: negative principal component values for body size and food availability correspond to larger bodies and higher food availability.

5.5 Discussion

Our results support the hypothesis that variation in inter- and intra-specific competition for food resources drives inter-island divergence in the body size of LBWE and OBSB.

The findings suggest that there are species-specific differences in the selective forces driving divergence. For example, divergence in LBWE body size was primarily driven by intraspecific competition, as it was best predicted by variation in the availability of food resources. These results provide some support for the dominance hypothesis. Conversely, our results suggest that divergence in OBSB body size was driven by variation in food availability and the presence/absence of the WWWE; providing limited support for the niche variation hypothesis.

While variation in LBWE body size showed no relationship with the density of LBWE, the species' body size did increase as the availability of food decreased. These results support the dominance hypothesis (MacArthur, 1961; Kikkawa, 1980; Scott et al., 2003), suggesting that as the availability of resources decreased, the level of intraspecific competition increased and thus there was greater selection for large body size as it confers a competitive advantage in agonistic interactions (Kikkawa, 1980). As invertebrates make up the largest proportion of LBWE diet, it is likely that data on the density of available invertebrate food sources would explain an even greater amount of variation in LBWE body size. However, evidence suggests that the density of fruit and nectar resources correlates with invertebrate densities to a certain degree (e.g. Abbas et al., 2014). LBWE also displayed higher potential for intraspecific interactions as they occurred in flocks of three or more individuals more often (31% of occurrences) than OBSB (8% of occurrences). This further supports intraspecific competition as the selective agent of divergence in LBWE body size.

Interestingly, LBWE body size also varied (to a lesser degree) with the biomass of ecologically similar, potential competitors; OBSB and ISMON. The effects of these species on LBWE body size were similar in strength but differed in direction. As ISMON increased in biomass, LBWE became smaller. Conversely, as OBSB biomass increased, LBWE became larger. OBSB were recorded as the dominant competitors in LBWE-OBSB agonistic interactions, while no agonistic interactions were recorded for LBWE and ISMON. It is difficult to discern the exact nature of these relationships but it may be that as OBSB increased in biomass, the level of competition between them and LBWE increased, thus potentially putting selective pressure on remaining LBWE to become larger and more competitive (whether that be inter- or intra-specifically). The increase in ISMON biomass which correlated with a decrease LBWE size may simply reflect an increase in food availability for both species. Increased food availability suggests that a larger avian biomass could be supported, which, in turn, would result in lower intraspecific competition

and thus reduced selection for large body size. However, we can only speculate as to the exact causes of these correlations as we do not have any data on the extent to which LBWE and ISMON competed indirectly for resources, if at all.

Our results suggest that variation in the level of interspecific competition and food availability have driven body size variation in OBSB across the Wakatobi archipelago. OBSB decreased in body size where the WWWE was present, suggesting the two species likely competed directly and/or indirectly for resources. This relationship provides some support for the niche variation hypothesis; as predicted by this hypothesis, OBSB decreased in size in the presence of a new, larger competitor. This suggests WWWE have not only driven ecological character displacement of LBWE on Wangi-wangi (see Chapter 4) but also divergence in OBSB size. These results suggest that the WWWE have driven community-wide character displacement among the avifauna of Wangi-wangi (Strong et al., 1979; Dayan and Simberloff, 2005). However, we do not know if the decrease in OBSB body size on Wangi-wangi corresponds to a narrowing of niche breadth as predicted (Grant, 1968) and we have no evidence of direct or indirect competition between WWWE and OBSB. While the displacement of the LBWE by the WWWE could also have had knock-on effects on the OBSB, the exact mechanism driving divergence in OBSB body size remains unclear.

explain why below doesnt work and why its strange... Our results reject the dominance hypothesis as an explanation for OBSB body size variation. Body size and density were not significantly correlated and body size did not vary significantly with the density of nectar resources, the primary food source of OBSB. This suggests that access to resources were not driving differences in the body size of OBSB.

Unlike the pattern observed in LBWE, as food availability increased, OBSB increased in body size; a pattern that is not in accordance with the dominance hypothesis. While we cannot explain this pattern, the disparity between the selective mechanisms driving body size variation in LBWE and OBSB may be linked to differences in the species behaviour and ecology. For example, OBSB were sexually dimorphic, with larger males than females across all islands, while LBWE were not. Thus, male and female OBSB probably had different energetic demands (Andersson, 1994). Furthermore, it may be that sexual selection is the dominant selective force acting upon OBSB body size. However, one would imagine that female selection for larger males would be linked to food resource acquisition. While there may be sex-specific drivers of body size variation in OBSB, we do not have sex-specific density data and thus cannot accurately test such hypotheses

here.

LBWE had a generalist diet, consuming invertebrates primarily, but also fruit and nectar, while the OBSB diet was predominantly comprised of nectar, supplemented with invertebrates. In comparison to other islands, both species exhibited variation in the composition of their diets on Binongko and this is likely to reflect a reduction in the density of fruit and nectar resources on Binongko in May (as inferred from our comparisons of resource densities on Tomia between May and July). Furthermore, variation in LBWE diet on Wangi-wangi is likely to be related to competition with the WWWE (see Chapter 3).

Our results suggest competitive interactions for resources, within and between species, drive body size divergence in island birds. Our results suggest there is a strong link between the density of food resources and levels of intraspecific competition, which drive variation in body size. Furthermore, inter- and intra-specific competition may drive phenotypic divergence to varying degrees depending on the ecology and reproductive biology of the species in question. While our results cannot rule out alternate mechanisms of phenotypic evolution, such as random genetic drift, previous studies suggest that such processes are insufficient to explain morphological divergence in island birds (Clegg et al., 2002b). Together, our results provide further evidence of the powerful role that competition plays in driving and maintaining phenotypic divergence among populations.

6.1 Avian systematics and speciation

The systematics of the birds of Asia remain in need of major reassessment (BirdLife International, 2013a). The necessity of resolving these evolutionary relationships has implications not just for our understanding of the patterns and processes of diversification but also for targeted conservation (Bickford et al., 2007). This issue can only be addressed with the application of integrative taxonomic methods and modern species delimitation criteria. Taxonomists currently have a wealth of available tools, such as DNA analysis and geometric morphometrics, and resources, such as GenBank (Benson et al., 2013), the Barcode of Life Database (Ratnasingham, 2007) and museum collections. Our understanding of what defines a species has progressed rapidly in recent decades and the application of the general lineage concept (de Queiroz, 2007) greatly complements the use of integrative analysis; examining multiple lines of evidence to deduce taxonomic classifications and evolutionary relationships (e.g. Tobias et al., 2010). The findings of my research support these claims. Focussing only on south-eastern Sulawesi and combining molecular and phenotypic data, I reclassified one species, the Wakatobi flowerpecker (*Dicaeum kuehni*), as a monotypic species endemic to the Wakatobi Islands (Chapter 2), and identified the olive-backed sunbirds of the Wakatobi (*Cinnyris jugularis infrenatus*) as an incipient species (Chapter 3). Furthermore, integrative analyses have determined that lemon-bellied white-eye populations from the Wakatobi Islands (*Zosterops chloris flavissimus*) are a distinct species, endemic to the Wakatobi archipelago (Kelly, D.J. et al., unpublished data).

Together, these results, the discovery of further cryptic avian diversity (Lohman et al., 2010) and the lack of ornithological research in Sulawesi suggest there are many taxonomic and systematic relationships to be resolved, with numerous species awaiting description. More importantly, the results from Chapter 2 and Chapter 3 suggest that studies combining data from multiple sources can successfully identify cryptic diversity and resolve taxonomic relationships. The multi-disciplinary taxonomic methods advocated here are

not exclusive to birds and can be applied to all taxonomic groups, across all regions of the globe.

6.1.1 *Patterns of diversification in the Wakatobi archipelago*

It is intriguing to consider why the sunbirds of the Wakatobi Islands have not diverged from their mainland counterparts to the same extent as flowerpecker (and lemon-bellied white-eye) populations. It could simply be that the *Dicaeum kuehni* colonised the Wakatobi before *Cinnyris jugularis* and have thus been diverging for longer. While there is insufficient fossil and geological data to time-calibrate my phylogenetic analyses, if I were to adopt the probably over-simplified "rule" of 2% divergence per million years in avian, protein-coding mitochondrial DNA (Lovette, 2004), the interpretation of my results would suggest the Wakatobi flowerpeckers colonised the Wakatobi archipelago c. 250,000-400,000 years before the sunbirds. While this may explain the observed differences in extent of genetic divergence, it may also be that flowerpeckers (Dicaeidae) diversify quicker than sunbirds (Nectariniidae). A study of genetic variation in bird populations across the Solomon Islands found results similar to mine; the flowerpeckers there (*Dicaeum aeneum*) showed high levels of genetic divergence between populations while *C. jugularis* populations did not (Smith et al., 2007). The application of multi-locus phylogenetic studies, calibrated using geological and fossil data, would provide further insight with regard to colonisation times and diversification rates.

The results from the phylogenetic analyses of both Wakatobi sunbirds and flowerpeckers suggest there may be a geographical divide among populations. This apparent divide separates populations north and south of the channel between Kaledupa and Tomia, the largest distance (18 km) between any one of the four Wakatobi islands and its nearest neighbour. This separation is most pronounced in the flowerpecker phylogeny (see Chapter 2, Figure 2.2), although there are no samples from Binongko or Runduma in this analysis. There is a suggestion of a similar divide among sunbirds but the support is much weaker and the signal is less clear (see Chapter 3, Figure 3.3). A more defined separation among flowerpecker populations may, again, be attributed to the earlier colonisation or higher diversification rate of flowerpeckers. Sampling from all islands and sequencing of both mitochondrial and nuclear DNA would provide increased insight into the population genetics of Wakatobi populations (e.g. see Milá et al., 2010).

6.2 Competitive interactions drive phenotypic diversification

It has long been proposed that competition plays a major role in divergence and speciation of organisms, yet robust empirical examples are lacking (Pfennig and Pfennig, 2012b). The results of Chapter 4 and Chapter 5 demonstrate that competitive biotic interactions among organisms can drive and maintain diversification via divergent and directional selection.

Chapter 4 shows that interspecific competitive interactions can drive adaptive divergence in resource exploiting traits, providing support for the theory of ecological character displacement. In this chapter, it was determined that *Z. chloris* had been displaced in bill size and feeding ecology where it co-exists with the larger, congeneric competitor, the 'Wangi-wangi' white-eye. To my knowledge, this is the first case of ecological character displacement described in white-eyes (Zosteropidae), a remarkable family of birds that have one of the highest diversification rates among vertebrates (Moyle et al., 2009) and have colonised more islands than any other passerine family (Moreau, 1964; van Balen, 2008). White-eyes from the *Zosterops* genus are found in sympatry with congeners more commonly on islands than on mainlands (van Balen, 2008). Thus, in consideration of my findings and the adaptive, divergent nature of white-eyes, I believe white-eyes are an ideal group for further empirical research into ecological character displacement and other competitively-mediated evolutionary processes. Additionally, I demonstrated the potential application of stable isotope analysis to the study of competitively-mediated divergence. When using stable isotope analysis to infer an organism's feeding ecology, I recommend that future studies also run isotopic analyses of a diversity of known and potential prey types. Bayesian isotopic mixing models (Parnell et al., 2013) can then accurately determine the proportional contribution of prey types to overall diet.

My analyses of body size variation (Chapter 5) implicate both inter- and intra-specific competition for resources as drivers of body size divergence in island passerines. It seems that interspecific competition has driven body size divergence in *C. jugularis*, as, in the presence of the 'Wangi-wangi' white-eye, *C. jugularis* were smaller. *Cinnyris jugularis* also shows some morphological divergence on the island of Hoga, where both white-eye species are absent (Kelly, D.J. et al, unpublished data). This divergence would be an interesting avenue for future research. Conversely, I concluded that intraspecific competition was the primary driver of body size divergence in *Z. chloris*. This suggests that the strength of

selective pressures resulting from competitive interactions will vary according to ecological traits of a species. For example, if a species is sexually dimorphic, the strength of sexual selection could outweigh any selective pressure from competitive interactions. My work has shown that by combining behavioural, ecological and morphological data, one can determine how competitive ecological interactions drive divergence. However, our results are limited by a lack of data on the density of available invertebrate resources. Future studies need to develop sampling protocols to quantify the density and/or biomass of available invertebrate food resources and incorporate the time required for such detailed work into their research timetables.

The behavioural and ecological studies in Chapter 4 and Chapter 5 were carried out at a time of year when food resources were likely to be plentiful. Data collection on competitive interactions and the availability of food resources took place after the peak rainfall season (April to June) in South-east Sulawesi (Whitten et al., 2002). Feather samples used in stable isotope analysis were collected during the same sampling periods but reflect the feeding ecology of birds at the time the feathers were grown (Inger and Bearhop, 2008). Thus, lemon-bellied white-eye and 'Wangi-wangi' white-eye feather samples, used in Chapter 4, most likely reflect the feeding ecology of individuals in September and October, during their post-breeding moult (Mees, 1961; van Balen, 2008). As these behavioural, resource and isotopic data are all representative of a period of plentiful food resources, I advise that future studies take place during periods when resources are scarce (e.g. when conditions become very dry during late October and November; Whitten et al., 2002). This might shed further light on the extent of direct and indirect competition for food resources, as well as niche overlap, within and between species. Data on the density of food resources during these periods would be of great use here. Grant and Grant (2008) have demonstrated the usefulness of such comparisons, showing that when resources are bountiful, *Geospiza* finches overlap significantly in resource use with little interspecific competition, but diverge in resource use as a result of competition when resources are scarce.

No single line of evidence will be sufficient to detect or describe a case of ecological character displacement or other competitively-mediated evolutionary process. Therefore, future studies need to combine data on the morphology, behaviour and feeding ecology (via direct behavioural observations and isotopic inferences) of study species with data on the density and diversity of available food resources.

6.3 Future prospects

6.3.1 *Diversification in Sulawesi*

The variation in environmental conditions within the Sulawesi region, with its complex climate, topology and geological history (Whitten et al., 2002), promote the separation and diversification of lineages (Whittaker and Fernández-Palacios, 2007). This is exemplified in the discovery of many new bird species from offshore islands (e.g. Rasmussen et al., 2000; Indrawan and Somadikarta, 2004; Indrawan et al., 2008) and the distribution of birds such as the scaly-headed white-eye (*Lophozosterops squamiceps*), whose subspecies are separated among, and confined to, many of Sulawesi's mountain ranges (van Balen, 2008). This pattern is also observed in other taxonomic groups, such as the distribution of macaque (*Macaca*) species and subspecies which are separated by high mountain ranges and as well as short oceanic distances (Fooden, 1969). I determined in Chapter 2 and Chapter 3 that, despite being separated by only 27 km of ocean, Wakatobi flowerpecker and sunbird lineages were genetically and morphologically distinct from those on Sulawesi. Thus, there are excellent opportunities throughout the Sulawesi region to resolve taxonomic relationships and study the broad scale patterns and processes of diversification. Such research must employ integrative taxonomic tools and extensively sample from the region's peninsulas, mountains and islands.

6.3.2 *Eco-evolutionary dynamics*

Evolutionary biology has progressed markedly in recent decades with the discovery that evolutionary change can be observed across ecological time scales (e.g. Grant and Grant, 2006); leading to the establishment of the field of eco-evolutionary dynamics. While there are many exceptional laboratory studies of rapid evolution in response to changing ecological conditions (e.g. Bolnick, 2001), the most powerful come from studies of wild animals, such as silvereyes (*Zosterops lateralis*) on Heron Island, Australia (see Kikkawa, 1980; Clegg and Owens, 2002) and studies of Darwin's finches (*Geospiza*) in the Galapagos (see Grant and Grant, 2008). Thus, to further understand how ecological interactions among organisms truly drive diversification, more long term studies are needed.

The Wakatobi Islands are currently thought to host three endemic bird species; the Wakatobi flowerpecker (Chapter 2), the 'Wangi-wangi' white-eye, and the lemon-bellied white-eye populations which are to be reclassified (Kelly, D.J. et al., unpublished data; see above). The islands also host an incipient species (Chapter 3) and have provided multiple

cases of competitively-mediated phenotypic divergence (Chapters 4 and 5). Thus, the Wakatobi archipelago has great potential to serve as a 'living lab' in which to study the patterns and processes of diversification through time. This would require commitment to further, regular sampling of bird populations (e.g. morphological, behavioural and tissue sampling) and environmental conditions (e.g. resource sampling) in the long term. As my research has already shown the value of short term study on the Wakatobi archipelago, there is great potential for long term study in the area. As environmental conditions change in response to climate change (Hannah et al., 2013), such a long term study could determine the species traits and environmental conditions that initially facilitate adaptive divergence. This could also further describe the mechanisms that drive and maintain divergence and ultimately lead to speciation. Additionally, it could be used to track the effects of climate change by monitoring the colonisation or extinction of species in the Wakatobi archipelago.

If one were to conduct a long term study of the Wakatobi avifauna, one should monitor the future interactions of lemon-bellied white-eye and 'Wangi-wangi' white-eye on Wangi-wangi. This would provide further insight into the process of ecological character displacement and the adaptive evolutionary response of lemon-bellied white-eye to this novel competitive environment. Furthermore, this could determine whether the divergence of lemon-bellied white-eye on Wangi-wangi is at an early stage and whether they will continue to survive, adapt and diverge in morphology and feeding ecology from neighbouring Wakatobi populations. The consequences such divergence may have on processes such as mate recognition and assortative mating could also be studied. One could also potentially witness species colonising new islands and record the adaptive response of the local avifauna. The beauty of such long term studies is that one cannot entirely predict what biotic and abiotic changes will occur and how they will affect the species and communities in question.

6.3.3 *Testing for competitively-mediated divergence*

While the results of Chapter 4 provide support for the theory of ecological character displacement, the ubiquity of this process remains in question (see Stuart and Losos, 2013). Although there are a large number studies in support of ecological character displacement, the majority rely on very few taxa (e.g. anoles lizards, sticklebacks, Darwin's finches and spadefoot toads) and there are a lack of robust studies that can rule out alternative

explanations (see Stuart and Losos, 2013). Furthermore, there have been relatively few empirical tests of the niche-variation hypothesis and the dominance hypothesis, both of which suggest that competitively-mediated selection explains morphological shifts in island fauna. Thankfully, an abundance of modern sampling techniques and analytical tools, as well as data sources (e.g. citizen science programmes; Cooper et al., 2014), are now available to evolutionary ecologists, whether to conduct long term studies or to investigate individual questions. I therefore recommend that, for more rigorous testing of the role of competition in micro-evolutionary change, future studies combine morphological sampling, DNA and stable isotope analysis, with detailed behavioural studies of individuals (e.g. via colour-ringing programmes or the use of electronic tags such as radio-frequency identification tags; Bonter, 2011). For example, it would be very interesting to compare the relationships between morphology, behavioural ecology and isotopic niche width of Wakatobi populations to their close relatives on mainland Sulawesi. Such a comparison, a test of the niche variation hypothesis, would provide further insight into how populations diverge in response to decreased competition and/or predation.

As Earth's climate changes, many species are likely to experience range expansions, resulting in the creation of novel competitive environments and interactions (Hoffmann and Sgró, 2011). Theoretical models suggest that interspecific resource competition may limit species' evolutionary responses to climate change by inhibiting the rates of adaptation and leading to increased extinction risk (De Mazancourt et al., 2008; Johansson, 2008; Pigot and Tobias, 2013). Empirical studies support these claims and have shown that competition for niche space can slow the rate of diversification and inhibit adaptation (e.g. Price et al., 2014). Therefore, gaining further insight into how competitive interactions drive evolutionary change, as well as the traits that make a species more or less likely to adapt to intense competition, are essential avenues for future research and will have implications for the conservation of biodiversity. Competition has been, and will continue to be, a major selective agent in the generation, maintenance and loss of biodiversity.

Bibliography

- Abbas M, Klein AM, Ebeling A, Oelmann Y, Ptacnik R, et al. (2014) Plant diversity effects on pollinating and herbivorous insects can be linked to plant stoichiometry. *Basic and Applied Ecology* 15: 169 – 178.
- Abzhanov A, Kuo W, Hartmann C, Grant B, Grant P, et al. (2006) The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature* 442: 563–567.
- Abzhanov A, Protas M, Grant BR, Grant PR, Tabin CJ (2004) Bmp4 and Morphological Variation of Beaks in Darwin's Finches. *Science* 305: 1462–1465.
- Adams D, Rohlf F (2000) Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences of the United States of America* 97: 4106–4111.
- Adler GH, Levins R (1994) The Island Syndrome in Rodent Populations. *The Quarterly Review of Biology* 69: 473–490.
- Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.
- Alatalo RV, Gustafsson L (1988) Genetic component of morphological differentiation in coal tits under competitive release. *Evolution* 42: 200–203.
- Aldridge HDJN, Rautenbach IL (1987) Morphology, Echolocation and Resource Partitioning in Insectivorous Bats. *Journal of Animal Ecology* 56: 763–778.
- Alström P, Rasmussen P, Olsson U, Sunberg P (2008) Species delimitation based on multiple criteria: the Spotted Bush Warbler *Bradypterus thoracicus* complex (Aves: Megaluridae). *Zoological Journal of the Linnean Society* 154: 291–307.
- Andersson M (1994) Sexual selection. Princeton University Press, New Jersey.
- Ashton KG (2002) Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography* 11: 505–523.
- Aubret F, Shine R (2009) Genetic assimilation and the postcolonization erosion of phenotypic plasticity in island Tiger Snakes. *Current Biology* 19: 1932–1936.
- Barbara B, Brent H, Brown ET, Russell G (2013) Divergent selection on bill morphology contributes to nonrandom mating between swamp sparrow subspecies. *Animal Behaviour* 86: 467–473.
- Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. URL <http://CRAN.R-project.org/package=lme4>, r package version 1.1-7.
- Beauchamp G (2010) Relaxed predation risk reduces but does not eliminate sociality in birds. *Biology letters* 6: 472–474.
- Begon M, Townsend CR, Harper JL (2006) *Ecology: From Individuals to Ecosystems* (4th ed.). Oxford, UK: Blackwell Publishing.
- Beheregaray L, Caccone A (2007) Cryptic biodiversity in a changing world. *Journal of Biology* 6: 9.1–9.5.
- Benson D, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman D, et al. (2013) GenBank. *Nucleic Acids Research* 41: D36–42.

- Bergmann C (1847) Ueber die verhältnisse der warmeconomie der thiere zu ihrer grosse. Gottinger studien 3: 595–708.
- Bibby C, Burgess N, Hill D, , Mustoe S (2000) Bird Census Techniques, 2nd ed. 2nd ed., London: Academic Press.
- Bickford D, Lohman D, Sodhi N, Ng P, Meier R, et al. (2007) Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22: 148–155.
- BirdLife International (2013a) Data zone: taxonomy. Available: <http://www.birdlife.org/datazone/info/taxonomy>. Accessed: 17 August 2013.
- BirdLife International (2013b) Endemic bird area factsheet: Kepulauan Wakatobi. Available: <http://www.birdlife.org/datazone>. Accessed: 12 August 2013.
- Blair C, Abrahamson W, Jackman J, Tyrrell L (2005) Cryptic speciation and host-race formation in a purportedly generalist tumbling flower beetle. Evolution 59: 304–316.
- Blondel J (2000) Evolution and ecology of birds on islands: trends and prospects. Vie et Milieu 50: 205–220.
- Blondel J, Chessel D, Frochot B (1988) Bird Species Impoverishment, Niche Expansion, and Density Inflation in Mediterranean Island Habitats. Ecology 69: 1899–1917.
- Bolnick D (2001) Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. Nature 410: 463–466.
- Bolnick D, Ingram T, Stutz W, Snowberg L, Lau O, et al. (2010) Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. Proceedings of the Royal Society B: Biological Sciences 277: 1789–1797.
- Bolnick DI, Araújo MS (2011) Partitioning the relative fitness effects of diet and trophic morphology in the threespine stickleback. Evolutionary Ecology Research 13: 439–459.
- Bolnick DI, Paull JS (2009) Morphological and dietary differences between individuals are weakly but positively correlated within a population of threespine stickleback. Evolutionary Ecology Research 11: 1217–1233.
- Bonter ES D N & Bridge (2011) Applications of radio frequency identification (RFID) in ornithological research: a review. Journal of Field Ornithology 82: 1–10.
- Brelsford A, Irwin DE (2009) Incipient speciation despite little assortative mating: the yellow-rumped warbler hybrid zone. Evolution 63: 3050–3060.
- Brown W, Wilson E (1956) Character Displacement. Systematic Zoology 5: 49–64.
- Chai P, Kirwan G (2014) Bee Hummingbird (*Mellisuga helenae*). Barcelona (retrieved from <http://www.hbw.com/node/55653> on 7 September 2014): Lynx Edicions.
- Cheke R, Mann C (2008a) Family Dicaeidae (Flowerpeckers). In: del Hoyo, J. and Elliott, A. and Christie, D. A. (eds). Handbook of the birds of the world Volume 13: Penduline-tits to Shrikes. Barcelona: Lynx Edicions, 350–389 pp.
- Cheke R, Mann C (2008b) Family Nectariniidae (Sunbirds). In: del Hoyo, J. and Elliott, A. and Christie, D. A. (eds). Handbook of the birds of the world Volume 13: Penduline-tits to Shrikes. Barcelona: Lynx Edicions, 196–321 pp.
- Chesser R (1999) Molecular systematics of the rhinocryptid genus *Pteroptochus*. Condor 101: 439–446.
- Clegg S, Frentiu F, Kikkawa J, Tavecchia G, Owens I (2008) 4000 years of phenotypic change in an island bird: heterogeneity of selection over three microevolutionary timescales. Evolution 62: 2393–2410.

- Clegg S, Owens I (2002) The 'island rule' in birds: medium body size and its ecological explanation. *Proceedings of the Royal Society B: Biological Sciences* 269: 1359–1365.
- Clegg SM, Degnan SM, Kikkawa J, Moritz C, Estoup A, et al. (2002a) Genetic consequences of sequential founder events by an island-colonizing bird. *Proceedings of the National Academy of Sciences of the United States of America* 99: 8127–8132.
- Clegg SM, Degnan SM, Moritz C, Kikkawa J, Estoup A, et al. (2002b) Microevolution in island forms: the roles of drift and directional selection in morphological divergence of a passerine bird. *Evolution* 56: 2090–2099.
- Coates B, Bishop K (1997) A guide to the birds of Wallacea: Sulawesi, the Moluccas and Lesser Sunda Islands, Indonesia. Australia: Dove Publications Pty. Ltd.
- Coates B, Dutson G, Filardi C, Clement P, Gregory P, et al. (2008) Family Monarchidae (Monarch-flycatchers). In: del Hoyo, J. and Elliott, A. and Christie, D. A. (eds). *Handbook of the birds of the world Volume 11: Old World Flycatchers to Old World Warblers*. Barcelona: Lynx Edicions.
- Conservation International (2014) Priority Areas - Wallacea. Available: <http://www.conservation.org>. Accessed: 8 August 2013.
- Cooper C, Shirk J, Zuckerberg B (2014) The invisible prevalence of citizen science in global research: migratory birds and climate change. *PLoS ONE* 9: e106508.
- Darwin C (1859) *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London: John Murray.
- Darwin CR, Wallace AR (1858) On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection. *Journal of the Proceedings of the Linnean Society of London: Zoology* 3: 45–50.
- Dayan T, Simberloff D (2005) Ecological and community-wide character displacement: the next generation. *Ecology Letters* 8: 875–894.
- De Mazancourt C, Johnson E, Barraclough TG (2008) Biodiversity inhibits species' evolutionary responses to changing environments. *Ecology Letters* 11: 380–388.
- de Queiroz K (2007) Species Concepts and Species Delimitation. *Systematic Biology* 56: 879–886.
- del Hoyo J, Elliott A, Christie DA (2008) *Handbook of the Birds of the World. Volume 13: Penduline-tits to Shrikes*. Barcelona: Lynx Edicions.
- Deméré T (2014) Family Balaenopteridae (Rorquals). Barcelona: Lynx Edicions.
- DeNiro M, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42: 495–506.
- Dennis A, Hellberg M (2010) Ecological partitioning among parapatric cryptic species. *Molecular Ecology* 19: 3206–3225.
- Dhondt A (2012) *Interspecific competition in birds*. Oxford: Oxford University Press.
- Drummond A, Ho S, Phillips M, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4:e88.
- Elliott A (1994) Family Megapodiidae (Megapodes). Barcelona: Lynx Edicions.
- Fairbanks RG (1989) A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. *Nature* 342: 637–642, URL <http://dx.doi.org/10.1038/342637a0>.
- Fjeldsa J, Irestedt M, Ericson PGP, Zuccon D (2010) The Cinnamon Ibon *Hypocryptadius cinnamomeus* is a forest canopy sparrow. *Ibis* 152: 747–760.

- Fooden J (1969) Taxonomy and evolution of the monkeys of Celebes. *Bibliotheca primatologica* 10: 1–148.
- Foster JB (1964) Evolution of Mammals on Islands. *Nature* 202: 234–235.
- Funk W, Caminer M, Ron S (2012) High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B: Biological Sciences* 279: 1806–1814.
- Gause G (1934) *The Struggle for Existence*. Baltimore: Williams & Wilkins.
- Gladfelter WB, Johnson WS (1983) Feeding niche separation in a guild of tropical reef fishes (Holocentridae). *Ecology* 64: 552–563.
- Glaw F, Kohler J, Townsend TM, Vences M (2012) Rivaling the World's Smallest Reptiles: Discovery of Miniaturized and Microendemic New Species of Leaf Chameleons (Brookesia) from Northern Madagascar. *PLoS ONE* 7: e31314.
- Grant B, Grant P (2010) Songs of Darwin's finches diverge when a new species enters the community. *Proceedings of the National Academy of Sciences of the United States of America* 107: 20156–20163.
- Grant P (1968) Bill size, body size, and the ecological adaptations of bird species to competitive situations on islands. *Systematic Zoology* 17: 319–338.
- Grant P (1972) Convergent and divergent character displacement. *Biological Journal of the Linnean Society* 4: 39–68.
- Grant P, Grant B (2008) Pedigrees, assortative mating and speciation in Darwin's finches. *Proceedings of the Royal Society B: Biological Sciences* 275: 661–668.
- Grant PR, Grant BR (1994) Phenotypic and genetic effects of hybridization in Darwin's finches. *Evolution* 48: 297–316.
- Grant PR, Grant BR (1995) Predicting microevolutionary responses to directional selection on heritable variation. *Evolution* 49: 241–251.
- Grant PR, Grant BR (2006) Evolution of Character Displacement in Darwin's Finches. *Science* 313: 224–226.
- Greenberg AJ, Moran JR, Coyne JA, Wu CI (2003) Ecological Adaptation During Incipient Speciation Revealed by Precise Gene Replacement. *Science* 302: 1754–1757.
- Gutiérrez E, Boria R, Anderson R (2014) Can biotic interactions cause allopatry? Niche models, competition, and distributions of South American mouse opossums. *Ecography* 37: 741–753.
- Hall R (2002) Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *Journal of Asian Earth Sciences* 20: 353–431.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hamilton TH (1961) The Adaptive Significances of Intraspecific Trends of Variation in Wing Length and Body Size Among Bird Species. *Evolution* 15: 180–195.
- Hannah L, Ikegami M, Hole DG, Seo C, Butchart SHM, et al. (2013) Global Climate Change Adaptation Priorities for Biodiversity and Food Security. *PLoS ONE* 8: e72590.
- Hardin G (1960) The Competitive Exclusion Principle. *Science* 131: 1292–1297.
- Hartert E (1903) On the birds collected on the Tukang-Besi Islands and Buton, south-east of Celebes, by Mr. Heinrich Kuhn. *Novitates Zoologicae* 10: 18–38.

- Hartert E (1920) Types of Birds in the Tring Museum. *Novitates Zoologicae* 28: 429.
- Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of Birds through DNA Barcodes. *PLoS Biology* 2: e312.
- Hoffmann A, Sgró C (2011) Climate change and evolutionary adaptation. *Nature* 470: 479–485.
- Holm S (1979) A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics* 6: 65–70.
- Hsu Y, Shaner P, Chang C, Ke L, Kao S (2014) Trophic niche width increases with bill size variation in a generalist passerine: a test of niche variation hypothesis. *Journal of Animal Ecology* 83: 450–459.
- Hutchinson G (1957) Concluding remarks. *Cold Spring Harbor Symposium on Quantitative Biology*. vol. 22, pp. 415–427.
- Indrawan M, Rasmussen PC, Sunarto (2008) A New White-Eye (*Zosterops*) from the Togian Islands, Sulawesi, Indonesia. *The Wilson Journal of Ornithology* 120: 1–9.
- Indrawan M, Somadikarta S (2004) A new hawk-owl from the Togian Islands, Gulf of Tomini, central Sulawesi, Indonesia. *Bulletin of the British Ornithologists' Club* 124: 160–171.
- Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. *Ibis* 150: 447–461.
- Isaac N, Mallet J, Mace G (2004) Taxonomic inflation: its influence on macroecology and conservation. *Trends in Ecology & Evolution* 19: 464–469.
- Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80: 595–602.
- Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO (2012) The global diversity of birds in space and time. *Nature* 491: 444–448.
- Johansson J (2008) Evolutionary responses to environmental changes: how does competition affect adaptation? *Evolution* 62: 421–435.
- Kaye S (1989) The Structure of Eastern Indonesia: an approach via gravity and other geophysical methods. Ph.D Thesis. University of London. Ph.D. thesis.
- Keller L, Grant P, Grant B, Petren K (2001) Heritability of morphological traits in Darwin's finches: misidentified paternity and maternal effects. *Heredity* 87: 325–336.
- Kelly D, Marples N (2011) Olive-backed Sunbird *Cinnyris jugularis* in southeast Sulawesi. *Birding Asia* 15: 15–16.
- Kelly DJ, Marples N (2010) Bird populations of the Wakatobi, pp. 149–170. New York: Nova Science Publishers.
- Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78: 1–27.
- Kelly SBA, Kelly DJ, Cooper N, Bahrnun A, Analuddin K, et al. (2014) Molecular and Phenotypic Data Support the Recognition of the Wakatobi Flowerpecker (*Dicaeum kuehni*) from the Unique and Understudied Sulawesi Region. *PLoS ONE* 9: e98694.
- Kerr KCR, Stoeckle MY, Dove CJ, Weigt LA, Francis CM, et al. (2007) Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes* 7: 535–543.
- Kikkawa J (1980) Winter survival in relation to dominance classes among silvereyes *Zosterops lateralis chlorocephala* of Heron Island, Great Barrier Reef. *Ibis* 122: 437–446.

- Lambert F (1998a) A new species of *Amaurornis* from the Talaud Islands, Indonesia, and a review of taxonomy of bush hens occurring from the Philippines to Australasia. *Bulletin of the British Ornithologists' Club* 118: 67–82.
- Lambert F (1998b) A new species of *Gymnocrex* from the Talaud Islands, Indonesia. *Forktail* 13: 1–6.
- Lambert F, Rasmussen P (1998) A new Scops Owl from Sangihe Island, Indonesia. *Bulletin of the British Ornithologists' Club* 118: 204–221.
- Lande R (2009) Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology* 22: 1435–1446.
- Linnaeus C (1770) *Systema natvrae per regna tria natvrae, secvndvm classes, ordines, genera, species cvm characteribvs, et differentiis*. Tomvs III. Vindobonae: Typis Ioannis Thomae.
- Lohman DJ, Ingram KK, Prawiradilaga DM, Winker K, Sheldon FH, et al. (2010) Cryptic genetic diversity in "widespread" Southeast Asian bird species suggests that Philippine avian endemism is gravely underestimated. *Biological Conservation* 143: 1885–1890.
- Lohman DJ, Prawiradilaga DM, Meier R (2009) Improved COI barcoding primers for Southeast Asian perching birds (Aves: Passeriformes). *Molecular Ecology Resources* 9: 37–40.
- Lomolino MV (1985) Body Size of Mammals on Islands: The Island Rule Reexamined. *The American Naturalist* 125: 310–316.
- Losos JB (2000) Ecological character displacement and the study of adaptation. *Proceedings of the National Academy of Sciences of the United States of America* 97: 5693–5695.
- Lovette IJ (2004) Mitochondrial dating and mixed support for the 2 percent rule in birds. *The Auk* 121: 1–6.
- Luo AR, Zhang YZ, Qiao HJ, Shi WF, Murphy RW, et al. (2010) Outgroup selection in the reconstruction: a case study of the family Halictidae (Hymenoptera: Apoidea). *Acta Entomologica Sinica* 53: 192–201.
- MacArthur R, Diamond J, Karr J (1972) Density Compensation in Island Faunas. *Ecology* 53: 330–432.
- MacArthur R, H Levins (1976) The limiting similarity, convergence, and divergence of co-existing species. *The American Naturalist* 110: 377–385.
- MacArthur RH (1961) Population Effects of Natural Selection. *The American Naturalist* 95: 195–199.
- MacArthur RH, Wilson EO (1967) *The Theory of Island Biogeography*. Princeton, N.J.: Princeton University Press.
- Maddison WP, Maddison D (2011) Mesquite: a modular system for evolutionary analysis. Version 2.75. Available: <http://mesquiteproject.org>.
- Martin P, Bateson P (1993) *Measuring Behaviour: An Introductory Guide* (2nd ed.). Cambridge: Cambridge University Press.
- Mayr E, Amadon D (1947) A review of the Dicaeidae. *American Museum Novitates* 1360: 1–32.
- McDonald P, Griffith S (2011) To pluck or not to pluck: the hidden ethical and scientific costs of relying on feathers as a primary source of DNA. *Journal of Avian Biology* 42: 197–203.

- McGuire J, Witt C, Remsen Jr JV, Corl A, Rabosky D, et al. (2014) Molecular Phylogenetics and the Diversification of Hummingbirds. *Current Biology* 24: 910–916.
- McKay B, Reynolds M, Hayes W, Lee D (2010) Evidence for the species status of the Bahama Yellow-throated Warbler (*Dendroica flavescens*). *The Auk* 127: 932–939.
- Mees GF (1961) A Systematic Review of the Indo-Australian Zosteropidae: Part II. *Zoologische Verhandelingen* 50: 1–168.
- Meier R, Zhang G, Ali F (2008) The Use of Mean Instead of Smallest Interspecific Distances Exaggerates the Size of the Barcoding Gap and Leads to Misidentification. *Systematic Biology* 57: 809–813.
- Meiri S, Cooper N, Purvis A (2008) The island rule: made to be broken? *Proceedings of the Royal Society B: Biological Sciences* 275: 141–148.
- Meiri S, Dayan T (2003) On the validity of Bergmann's rule. *Journal of Biogeography* 30: 331–351.
- Meiri S, Raia P, Phillimore AB (2011) Slaying dragons: limited evidence for unusual body size evolution on islands. *Journal of Biogeography* 38: 89–100.
- Melo M, Bowie RCK, Voelker G, Dallimer M, Collar NJ, et al. (2010) Multiple lines of evidence support the recognition of a very rare bird species: the Principe thrush. *Journal of Zoology* 282: 120–129.
- Melo M, Warren B, Jones P (2011) Rapid parallel evolution of aberrant traits in the diversification of the Gulf of Guinea white-eyes (Aves, Zosteropidae). *Molecular Ecology* 20: 4953–4967.
- Mendel G (1866) Versuche uber Pflanzen-Hybriden. *Verhandlungen des naturforschenden Vereines in Brunn* 4: 3–47 (in English in 1901, *J. R. Hortic. Soc.* 26: 1–32).
- Meyer AB, Wigglesworth LM (1898) *The birds of Celebes and neighbouring islands*. Berlin: Friedlander.
- Milá B, Warren B, Heeb P, Thebaud C (2010) The geographic scale of diversification on islands: genetic and morphological divergence at a very small spatial scale in the Mascarene grey white-eye (Aves: *Zosterops borbonicus*). *BMC Evolutionary Biology* 10: 158.
- Miles DB, Ricklefs RE (1984) The Correlation Between Ecology and Morphology in Deciduous Forest Passerine Birds. *Ecology* 65: 1629–1640.
- Milsom J, Ali J, Sudarwono (1999) Structure and collision history of the Buton continental fragment, eastern Indonesia. *AAPG Bulletin* 83: 1666–1689.
- Moczek A, Sultan S, Foster S, Ledon-Rettig C, Dworkin I, et al. (2011) The role of developmental plasticity in evolutionary innovation. *Proceedings of the Royal Society B: Biological Sciences* 278: 2705–2713.
- Moreau R (1964) White-eye. In: Thomson, A.L. (Ed.) *A new dictionary of birds*. New York, McGraw-Hill Book Co. Pp. 885–886.
- Moyle R, Filardi C, Smith C, Diamond J (2009) Explosive Pleistocene diversification and hemispheric expansion of a "great speciator". *Proceedings of the National Academy of Sciences of the United States of America* 106: 1863–1868.
- Myers N, Mittermeier R, Mittermeier C, da Fonseca G, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.

- Nakagawa S, Cuthill I (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews of the Cambridge Philosophical Society* 82: 591–605.
- Nash S (1993) *Sold for a Song - the Trade in Southeast Asian Non-CITES Birds*. Cambridge, UK: TRAFFIC International.
- Navarro J, Kaliontzopoulou A, Gonzalez-Solas J (2009) Sexual dimorphism in bill morphology and feeding ecology in Cory's shearwater (*Calonectris diomedea*). *Zoology* 112: 128–138.
- Newsome S, del Rio C, Bearhop S, Phillips D (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment* 5: 429–436.
- Nyári AS, Peterson AT, Rice NH, Moyle RG (2009) Phylogenetic relationships of flowerpeckers (Aves: Dicaeidae): Novel insights into the evolution of a tropical passerine clade. *Molecular Phylogenetics and Evolution* 53: 613–619.
- Padial J, De I (2010) A response to recent proposals for integrative taxonomy. *Biological Journal of the Linnean Society* 101: 747–756.
- Parnell A, Inger R, Bearhop S, Jackson A (2008) Package SIAR: Stable isotope analysis in R. <http://cranr-project.org/web/packages/siar/indexhtml> .
- Parnell A, Phillips D, Bearhop S, Semmens B, Ward E, et al. (2013) Bayesian stable isotope mixing models. *Environmetrics* 24: 387–399.
- Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source Partitioning Using Stable Isotopes: Coping with Too Much Variation. *PLoS ONE* 5: e9672.
- Pebesma E (2004) Multivariable geostatistics in S: the gstat package. *Computers & Geosciences* 30: 683–691.
- Peterson BJ, Fry B (1987) Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics* 18: 293–320.
- Pfennig D, Pfennig K (2012a) Development and evolution of character displacement. *Annals of the New York Academy of Sciences* 1256: 89–107.
- Pfennig D, Pfennig K (2012b) *Evolution's Wedge: Competition and the Origins of Diversity*. University of California Press, CA.
- Pigot A, Tobias J (2013) Species interactions constrain geographic range expansion over evolutionary time. *Ecology Letters* 16: 330–338.
- Podos J (2001) Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409: 185–188.
- Podos J, Nowicki S (2004) Beaks, adaptation, and vocal evolution in Darwin's finches. *Bioscience* 54: 501–510.
- Post D (2002) Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. *Ecology* 83: 703–718.
- Potvin DA, Mulder RA (2013) Immediate, independent adjustment of call pitch and amplitude in response to varying background noise by silvereyes (*Zosterops lateralis*). *Behavioral Ecology* 24: 1363–1368.
- Price T (2008) *Speciation in Birds*. CO, USA: Roberts and Co.
- Price T, Hooper D, Buchanan C, Johansson U, Tietze D, et al. (2014) Niche filling slows the diversification of Himalayan songbirds. *Nature* 509: 222–225.
- R Development Core Team (2013) *R: A language and environment for statistical computing*.

- Rasmussen P (1999) A new species of Hawk-Owl *Ninox* from North Sulawesi, Indonesia. *The Wilson Bulletin* 111: 457–464.
- Rasmussen P, Wardill J, Lambert F, Riley J (2000) On the specific status of the Sangihe White-eye *Zosterops nehrkorni*, and the taxonomy of the Black-crowned White-eye *Z. atrifrons* complex. *Forktail* 16: 69–80.
- Ratnasingham PDN S & Hebert (2007) BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes* 7: 355–364.
- Redfern C, Clark J (2001) *Ringersâ Manual* (4th ed.). British Trust for Ornithology, Thetford.
- Ricklefs RE (2012) Species richness and morphological diversity of passerine birds. *Proceedings of the National Academy of Sciences, USA* 109: 14482–14487.
- Rising J, Somers K (1989) The measurement of overall body size in birds. *The Auk* 106.
- Robinson-Wolrath SI, Owens IPF (2003) Large size in an island-dwelling bird: intraspecific competition and the Dominance Hypothesis. *Journal of Evolutionary Biology* 16: 1106–1114.
- Ronquist F, Huelsenbeck J (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, et al. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Rubinoff D, Holland B (2005) Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. *Systematic Biology* 54: 952–961.
- Schluter D (1995) Adaptive Radiation in Sticklebacks: Trade-Offs in Feeding Performance and Growth. *Ecology* 76: 82–90.
- Schluter D (2000a) Ecological Character Displacement in Adaptive Radiation. *The American Naturalist* 156: S4–S16.
- Schluter D (2000b) *The ecology of adaptive radiation*. Oxford: Oxford University Press.
- Schluter D, McPhail JD (1992) Ecological Character Displacement and Speciation in Sticklebacks. *The American Naturalist* 140: 85–108.
- Schoener T (1974) Resource partitioning in ecological communities. *Science* 185: 27–39.
- Scott S, Clegg S, Blomberg S, Kikkawa J, Owens I (2003) Morphological shifts in island-dwelling birds: the roles of generalist foraging and niche expansion. *Evolution* 57: 2147–2156.
- Setiadi M, McGuire J, Brown R, Zubairi M, Iskandar D, et al. (2011) Adaptive radiation and ecological opportunity in Sulawesi and Philippine fanged frog (*Limnonectes*) communities. *The American Naturalist* 178: 221–240.
- Shepard D (1968) A two-dimensional interpolation function for irregularly-spaced data. In: *Proceedings of the 23rd Association for Computing Machinery National Conference*, pp. 517–524.
- Slatkin M (1980) Ecological character displacement. *Ecology* 61: 163–177.
- Smith CE, Filardi CE, Fleischer RC (2007) Patterns of molecular and morphological variation in some Solomon Island land birds. *The Auk* 124: 479–493.
- Smith R, Silver E (1991) Geology of a Miocene collision complex, Buton, eastern Indonesia. *Geological Society of America Bulletin* 103: 660–678.

- Sodhi N, Koh L, Brook B, Ng P (2004) Southeast Asian biodiversity: an impending disaster. *Trends in Ecology & Evolution* 19: 654–660.
- Stamatakis A (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* .
- Stresemann E (1975) Ornithology, from Aristotle to the present. Cambridge, MA: Harvard University Press.
- Strong J Donald R, Szyska LA, Simberloff DS (1979) Test of Community-Wide Character Displacement Against Null Hypotheses. *Evolution* 33: 897–913.
- Stuart Y, Losos J (2013) Ecological character displacement: glass half full or half empty? *Trends in Ecology & Evolution* 28: 402–408.
- Stuessy TF, Takayama K, Sepulveda P (2012) Founder effects are invisible in endemic species of oceanic islands. *Journal of Biogeography* 39: 1565–1566.
- Sutherland NI WJ, Green R (2004) Bird ecology and conservation: handbook of techniques. Oxford: Oxford University Press.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731–2739.
- Taper M, Case T (1985) Quantitative genetic models for the coevolution of character displacement. *Ecology* 66: 355–371.
- Tavares ES, Baker AJ (2008) Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. *BMC Evolutionary Biology* 8: 81.
- Tobias J, Seddon N, Spottiswoode C, Pilgrim J, Fishpool L, et al. (2010) Quantitative criteria for species delimitation. *Ibis* 152: 724–746.
- van Balen S (2008) Family Zosteropidae (White-eyes). In: del Hoyo, J. and Elliott, A. and Christie, D. A. (eds). *Handbook of the birds of the world Volume 13: Penduline-tits to Shrikes*. Barcelona: Lynx Edicions, 402–485 pp.
- Van Valen L (1965) Morphological Variation and Width of Ecological Niche. *The American Naturalist* 99: 377–390.
- Waddington CH (1953) Genetic assimilation of an acquired character. *Evolution* 7: 118–126.
- Werle E, Schneider C, Renner M, Valke M, Fiehn W (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research* 22: 4354–4355.
- Werner T, Sherry T (1987) Behavioural specialisation in *Pinaroloxias inornata*, the Darwin's Finches of Cocos Island, Costa Rica. *Proceedings of the National Academy of Sciences* 84: 5506–5510.
- West J, Bowen G, Cerling T, Ehleringer J (2006) Stable isotopes as one of nature's ecological recorders. *Trends in Ecology & Evolution* 21: 408–414.
- Whittaker RJ, Fernández-Palacios JM (2007) Island biogeography: ecology, evolution, and conservation. Oxford University Press .
- Whitten T, Mustafa M, Henderson GS (2002) Ecology of Sulawesi. Tuttle Publishing, Vermont, USA.
- Will K, Mishler B, Wheeler Q (2005) The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* 54: 844–851.

- Wiltschko W, Munro U, Ford H, Wiltschko R (1998) Effect of a magnetic pulse on the orientation of silveryeyes, *Zosterops l. lateralis*, during spring migration. *Journal of Experimental Biology* 201: 3257–3261.
- Winkelmann K, Genner M, Takahashi T, Ruber L (2014) Competition-driven speciation in cichlid fish. *Nature communications* 5: 3.
- Winker K (2009) Reuniting Phenotype and Genotype in Biodiversity Research. *BioScience* 59: 657–665.
- Wolf J, Harrod C, Brunner S, Salazar S, Trillmich F, et al. (2008) Tracing early stages of species differentiation: ecological, morphological and genetic divergence of Galápagos sea lion populations. *BMC Evolutionary Biology* 8: 150.
- Worthy TH, Holdaway RN (2002) *The Lost World of the Moa*. Bloomington: Indiana University Press.
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. New York: Springer.

appendix a

Molecular and phenotypic data support the recognition of the Wakatobi flowerpecker (*Dicaeum kuehni*) from the unique and understudied Sulawesi region - Supplementary Material

Table a.1: Genbank accession numbers of COI and ND3 flowerpecker (Dicaeidae) sequences used in the flowerpecker phylogenetic analyses of Chapter 2. Taxonomic names shown are according to Genbank at time of download

Genus	Species	COI Accession Numbers	ND3 Accession Numbers
<i>Dicaeum</i>	<i>aeneum</i>	FJ473228	GQ145243
<i>Dicaeum</i>	<i>agile</i>	-	GQ145264
<i>Dicaeum</i>	<i>anthonyianthonyi</i>	-	GQ145241
<i>Dicaeum</i>	<i>aureolimbatum</i>	FJ473172	GQ145246
<i>Dicaeum</i>	<i>australe</i>	FJ473059	GQ145263; GU816805
<i>Dicaeum</i>	<i>bicolor</i>	-	GQ145242
<i>Dicaeum</i>	<i>celebicum</i>	JX067922; GQ145247	JX067913
<i>Dicaeum</i>	<i>chrysorrheum</i>	-	GQ145235
<i>Dicaeum</i>	<i>concolor</i>	-	GQ145255
<i>Dicaeum</i>	<i>cruentatum</i>	-	GQ145236
<i>Dicaeum</i>	<i>everetti</i>	-	GQ145265
<i>Dicaeum</i>	<i>eximium</i>	JQ174684;JQ174685	GQ145261
<i>Dicaeum</i>	<i>geelvinkianum</i>	-	GQ145237
<i>Dicaeum</i>	<i>hirundinaceum</i>	FJ473233	GQ145225
<i>Dicaeum</i>	<i>hypoleucum</i>	FJ473237	GQ145244
<i>Dicaeum</i>	<i>igniferum</i>	-	GQ145245
<i>Dicaeum</i>	<i>ignipectus</i>	-	GQ145262
<i>Dicaeum</i>	<i>kuehni</i>	JX067916;JX067921; JX067923;JX067924	JX067909;JX0679012; JX067914;JX067915
<i>Dicaeum</i>	<i>melanoxanthum</i>	-	GQ145228
<i>Dicaeum</i>	<i>monticolum</i>	-	GQ145257
<i>Dicaeum</i>	<i>nigrilore</i>	-	GQ145254
<i>Dicaeum</i>	<i>pygmaeum</i>	JQ174686	GQ145260
<i>Dicaeum</i>	<i>sanuinolentum</i>	-	GQ145239
<i>Dicaeum</i>	<i>triginostigma</i>	FJ473089; FJ473098; FJ473101; FJ473105; FJ473076; FJ473093; FJ473142; EU541455	GQ145224
<i>Dicaeum</i>	<i>tristrami</i>	-	GQ145258

Table a.2: Genbank accession numbers of COI and ND3 sunbird (Nectariniidae) sequences used in the flowerpecker phylogenetic analyses of Chapter 2. Taxonomic names shown are according to Genbank at time of download

Genus	Species	COI Accession Numbers	ND3 Accession Numbers
<i>Nectarinia</i>	<i>adelberti</i>	-	AY233991
<i>Nectarinia</i>	<i>alinae</i>	-	AY233992
<i>Nectarinia</i>	<i>bouvieri</i>	-	AY233993
<i>Nectarinia</i>	<i>erythrocerca</i>	-	AY233994
<i>Nectarinia</i>	<i>jugularis</i>	FJ473077:FJ473078; FJ473099; FJ473158; FJ473162:FJ473163; FJ473171; FJ473187:FJ473188; FJ473190; FJ473199	AY233995; GQ145229
<i>Nectarinia</i>	<i>kilimensis</i>	-	AY233996
<i>Nectarinia</i>	<i>minulla</i>	-	AY233997
<i>Nectarinia</i>	<i>olivacea</i>	FJ473232	AY233998; JF956832; GU816806
<i>Nectarinia</i>	<i>senegalensis</i>	AY233999	-
<i>Nectarinia</i>	<i>sperata</i>	EU541459	JF956833
<i>Nectarinia</i>	<i>talatala</i>	FJ473229	AY234000
<i>Nectarinia</i>	<i>venusta</i>	-	AY234001

appendix b

Incipient speciation of an island songbird lineage separated by a narrow oceanic barrier - Supplementary Material

Table b.1: Genbank accession numbers of COI and ND3 sunbird (Nectariniidae) sequences used in the sunbird phylogenetic analyses of Chapter 3. Taxonomic names shown are according to Genbank at time of download. All flowerpecker sequences used in these analyses are shown in Table a. *Cinnyrus jugularis* sequences generated in this study will be submitted to Genbank (and can currently be found on the attached data CD; see Appendix e) .

Genus	Species	COI Accession Numbers	ND3 Accession Numbers
<i>Nectarinia</i>	<i>adelberti</i>	-	AY233991
<i>Nectarinia</i>	<i>afra</i>	-	AY630975
<i>Nectarinia</i>	<i>alinae</i>	-	AY233992
<i>Nectarinia</i>	<i>balfouri</i>	-	AY235542
<i>Nectarinia</i>	<i>bouvieri</i>	-	AY233993
<i>Nectarinia</i>	<i>chalybea</i>	-	AY360976
<i>Nectarinia</i>	<i>chloropygia</i>	-	AY630977
<i>Nectarinia</i>	<i>coquereli</i>	-	AY235526
<i>Nectarinia</i>	<i>dussumieri</i>	-	AY235530
<i>Nectarinia</i>	<i>erythrocerca</i>	-	AY233994
<i>Nectarinia</i>	<i>jugularis</i>	FJ473077:FJ473078; FJ473099; FJ473106; FJ473116; FJ473158; FJ473162:FJ473163; FJ473169:FJ473170; FJ473171:FJ473188; FJ473234; FJ473236; FJ473238; FJ473240; JQ175525	AY233995; GQ145229
<i>Nectarinia</i>	<i>kilimensis</i>	-	AY233996
<i>Nectarinia</i>	<i>loveridgei</i>	-	AY630928:AY630931
<i>Nectarinia</i>	<i>mediocris</i>	-	AY630935; AY630939; AY630947; ZY360932
<i>Nectarinia</i>	<i>minulla</i>	-	AY233997
<i>Nectarinia</i>	<i>moreaui</i>	-	AY630948; AY630962:AY630963; AY630974
<i>Nectarinia</i>	<i>notata</i>	-	AY235537; AY235539:AY235540

Table b.2: Continued (from Table b.1): Genbank accession numbers of COI and ND3 sunbird (Nectariniidae) sequences used in the sunbird phylogenetic analyses of Chapter 3. Taxonomic names shown are according to Genbank at time of download. All flowerpecker sequences used in these analyses are shown in Table a.1. *Cinnyrus jugularis* sequences generated in this study will be submitted to Genbank (and can currently be found on the attached data CD; see Appendix e) .

Genus	Species	COI Accession Numbers	ND3 Accession Numbers
<i>Nectarinia</i>	<i>olivacea</i>	FJ473232	AY641382;AY641383; AY641387; AY641391; GU816806
<i>Nectarinia</i>	<i>preussi</i>	-	AY630979
<i>Nectarinia</i>	<i>regia</i>	-	AY630980
<i>Nectarinia</i>	<i>rufipennis</i>	-	AY360981
<i>Nectarinia</i>	<i>senegalensis</i>	-	AY233999; AY630978
<i>Nectarinia</i>	<i>sovimanga</i>	-	AY235510; AY235513; AY235517; AY235520; AY235523
<i>Nectarinia</i>	<i>sperata</i>	EU541459	JF956833
<i>Nectarinia</i>	<i>talatala</i>	FJ473229	AY234000
<i>Nectarinia</i>	<i>venusta</i>	-	AY234001
<i>Cinnyris</i>	<i>mariquensis</i>	FJ473131	-
<i>Cinnyris</i>	<i>pulchellus</i>	FJ473135	-
<i>Cinnyris</i>	<i>superbus</i>	-	GQ145249
<i>Cyanomitra</i>	<i>verticalis</i>	FJ473132	-
<i>Leptocoma</i>	<i>sericea</i>	FJ473226	-

Competitively-mediated micro-evolution: ecological character displacement in a pair of island-dwelling birds - Supplementary Material

Analysis of variance in LBWE bill length (adult birds, measured by NMM) revealed that bill length varied significantly across the archipelago ($F_{4, 394} = 15.85$, $p = 4.88e-12$; see Figure c.1). Moving south-east from the northernmost island of Wangi-wangi are the islands of Oroho, Kaledupa, Tomia and Binongko. Further to our comparisons of LBWE from Wangi-wangi and Oroho, post-hoc tests determined that LBWE bill length is significantly shorter on Wangi-wangi compared to Kaledupa ($t = -5.8405$, $df = 154.133$, $p = 8.9550e-08$), Tomia ($t = -7.2384$, $df = 126.538$, $p = 1.5524e-10$) and Binongko ($t = -5.469$, $df = 109.039$, $p = 5.7980e-07$). Note: these p-values have been corrected for multiple comparisons using Sequential Holm-Bonferroni corrections (Holm, 1979). LBWE bill size remains constant across Oroho, Kaledupa, Tomia and Binongko but is reduced on Wangi-wangi.

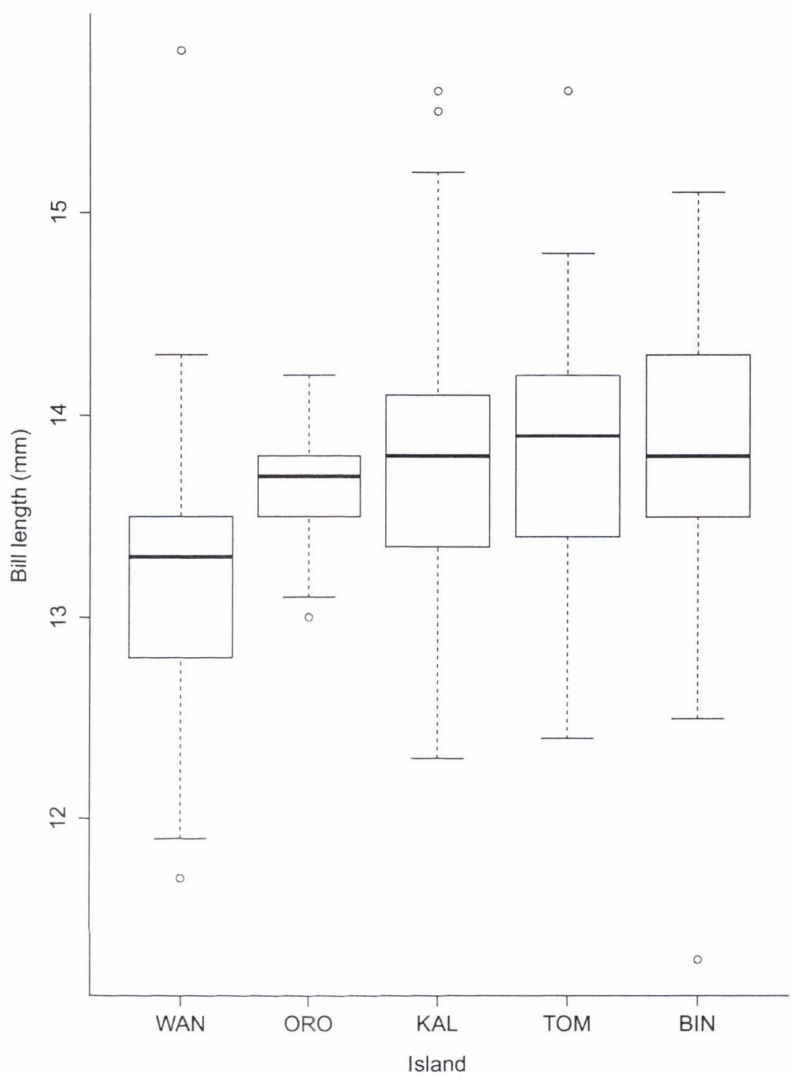


Figure c.1: A comparison of LBWE bill length across the Wakatobi archipelago. From north to south: Wangi-wangi (N = 75), Oroho (N = 9), Kaledupa (N = 10), Tomia (N = 162) and Binongko (N = 53).

Table c.1: Criteria of ecological character displacement satisfied in this study, as defined by Schluter and McPhail (1992). Lemon-bellied white-eye = LBWE; Wangi-wangi white-eye = WWWE.

Criterion	Supported?	Supporting data
Evidence of competition	Yes	Direct: behavioural observations showed WWWE attacking and displacing LBWE on Wangi-wangi. Indirect: behavioural and isotopic data show LBWE and WWWE exploited similar food resources and habitats.
Chance ruled out as an explanation	Yes	The observed change in LBWE bill length between Wangi-wangi and Oroho was greater than expected by chance (see Section 4.4 - Results). LBWE bill size was consistently similar across all of the Wakatobi islands, except on Wangi-wangi where the LBWE had shorter bills (in the presence of the WWWE; see Figure c.1).
Morphological differences reflected in resource use	Yes	Due to a lack of behavioural data from Oroho, we could not draw any comparisons in feeding behaviour between LBWE populations. While our isotopic data showed no difference in diet breadth or trophic position between LBWE populations, we did find significant overlap in the range of prey items taken and habitats occupied by LBWE and WWWE on Wangi-wangi. This suggests high potential for competition and we believe the decrease in LBWE bill size indicates a change to the consumption of smaller arthropod prey items and thus resource partitioning between the two species (see Discussion, Chapter 4)

Table c.2: Continued (from Table c.1): criteria of ecological character displacement satisfied in this study, as defined by Schluter and McPhail (1992). Lemon-bellied white-eye = LBWE; Wangi-wangi white-eye = WWWE.

Criterion	Supported?	Supporting data
Sites of sympatry do not differ greatly in environmental pressures	Yes	Neither LBWE body size (as indicated from wing length and weight) nor tarsus length varied significantly between Wangi-wangi and Oroho. This suggests levels of intraspecific competition and the foraging environment are similar between the islands (Miles and Ricklefs, 1984; Scott et al., 2003).
Genetic basis to phenotypic differences	Partially	While we have no genetic data to support phenotypic divergence in the LBWE of Wangi-wangi island, bill morphology is known to be highly heritable and controlled by single gene pathways in other passerine birds (see Discussion, Chapter 4)
Phenotypic differences are an adaptive evolutionary response	Partially	LBWE bill length is consistently similar across all the Wakatobi islands, except on Wangi-wangi Island where the LBWE and WWWE co-exist. This suggests that the observed phenotypic difference in LBWE on Wangi-wangi is an adaptive response and not simply a result of biased-colonisation or species sorting.

Competition for food resources drives body size divergence in island birds - Supplementary Material

Table d.1: Ethogram of bird behaviour used in field observations.

Behaviour	Code	Definition
Foraging	FR	Bird actively moving through vegetation
Foraging while calling	FC	Bird foraging (as above) while calling
Feeding on unknown resource	FD	Bird feeding but resources being consumed is unclear/not visible
Feeding on nectar	FN	Bird inserting bill into open corolla or base of flower
Feeding on invertebrates	FI	Bird consuming invertebrates
Feeding on fruit	FF	Bird consuming fruit
Perched	P	Bird perching in a stationary position
Perched calling	PC	Bird perching in a stationary position while calling
Perched singing	PS	Bird perching in a stationary position while singing
Preening	PR	Bird cleaning its own feathers using its bill
Allopreening	APR	Bird cleaning feathers of a conspecific using its bill
Allofeeding	AFD	Bird passing unidentified food resource to a conspecific
Allofeeding fruit	AFF	Bird passing fruit to a conspecific
Allofeeding invertebrates	AFI	Bird passing invertebrate prey to a conspecific
Flying	FLY	Bird flying across transect area
Out of sight	OOS	Bird moves into area where it cannot be clearly observed
Agonistic interaction	AI	Aggresive interactions two or more birds, opposing one another, whether intra- or inter-specifically
Beak scrape	BS	Bird scrapes beak on a twig or other surface
Hovering	HO	Bird hovering in flight around one particular area
Nest building	NB	Bird actively constructing a nest or collecting nest material
Other	O	Other behaviours which are not covered above (each must be noted and explained in full)

appendix e

Electronic appendix

Attached is a compact disc containing a copy of all the data used in this thesis, formatted chapter by chapter.

Ph.D. thesis:

Integrative avian taxonomy and the role of competition in the diversification of passerine birds

Author:

Seán B.A. Kelly

Summary:

It is well recognised that the avifauna of Asia are in major need of taxonomic reassessment. The Sulawesi region of Indonesia, renowned for its high levels of avian endemism, has been relatively overlooked by ornithological researchers. Combining genetic, phylogenetic and morphological analyses, I reassessed the taxonomic relationships of avian populations from peninsular South-east Sulawesi and the nearby Wakatobi Islands. These integrative taxonomic comparisons describe the flowerpeckers (Dicaeidae) of the Wakatobi Islands as a genetically and phenotypically distinct species. I therefore advocate the reclassification of these populations as *Dicaeum kuehni*, the Wakatobi flowerpecker. Furthermore, my results describe the olive-backed sunbird populations present on the Wakatobi (*Cinnyris jugularis infrenatus*) as an incipient species, diverging from its relatives on mainland Sulawesi. Together with the lack of study in the region, my results suggest that species richness and endemism in Sulawesi are underestimated and underscore the need for further integrative taxonomic research.

As the Wakatobi archipelago hosts divergent avian lineages, it offers unique opportunities to study the process of diversification in island birds. To gain further insight into how competitive interactions among organisms drive diversification, I investigated patterns of inter-island phenotypic divergence. I studied the behaviour, ecology and morphology of two ecologically similar white-eye species (Zosteropidae) in sympatry and allopatry. My results suggest that competition with the larger 'Wangi-wangi' white-eye (*Zosterops* sp.) has driven the adaptive divergence of lemon-bellied white-eye (*Z. chloris*) bill size and feeding ecology (i.e. ecological character displacement). Finally, I investigated whether inter-island divergence in *Z. chloris* and *C. jugularis* body size correlated with competition for resources. My results describe competition for resources as a major predictor of body size variation and suggest that inter- and intra-specific competition may exert different selection pressures depending on the species' ecology. These results further highlight the powerful role competitive interactions can play in phenotypic diversification.

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