

# Dispersal and speciation in the avian archipelago

*by*

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# Declaration

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# Summary

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Islands play a key role in both the evolution of new species and our understanding of that evolution. The colonisation of islands, followed by different levels of gene flow between populations, has produced a diverse array of bird species scattered around the world, from which generations of biologists have drawn their insights. Like the islands on which they have evolved, these birds are divided into an “archipelago” of populations of different shapes and sizes, with varying levels of isolation between them. Their fragile ecosystems are under increasing threat, however, and may be lost before their diversity is fully catalogued or the evolutionary processes that birthed it are understood.

Though island birds have informed many important theories in evolutionary biology and biogeography, our knowledge of them is subject to many shortfalls. We have not named all of the species that exist (“Linnean shortfalls”) and we do not know how species are distributed (“Wallacean shortfalls”). These shortfalls are particularly concerning when it comes to islands with highly endemic faunas, such as Madagascar and Sulawesi. In this thesis I have worked to help address some Linnean and Wallacean shortfalls in our knowledge of island birds, as all biogeographic theory relies on accurate knowledge of the biota itself. I have also explored the population structure of birds in the important biodiversity hotspot of Sulawesi and its surrounding regions, supplying insights into evolutionary processes. From there, I have used the patterns uncovered to refine certain concepts from biogeography, in particular our understanding of how differences in the dispersal ability of birds affect gene flow. This, in turn, drives patterns of speciation and thus the generation of biodiversity.

The first two data chapters of this thesis (Chapters 2 and 3) contribute new natural history and distribution data for a number of island bird species. Chapter 2 contains the first detailed inventory of the bird species of Ankobohobo Wetland in Madagascar, improving our knowledge of the distribution and conservation status of some threatened and endemic species. In Chapter 3 I provide evidence of cryptic sexual dimorphism in the Sulawesi Babbler (*Pellorneum celebense*), an understory bird endemic to Sulawesi and surrounding islands in Indonesia. Distribution and sexual dimorphism are two aspects of natural history that can help to inform biogeographic theory.

The core of the thesis is made up of three chapters (Chapters 4 to 6) that delve deeper into biogeographic patterns around Sulawesi and surrounding regions, using DNA sequencing and integrative taxonomy. Chapter 4 returns to the story of the Sulawesi Babbler, finding that some populations show notable differences in DNA and song, even though they have been connected by land in recent geological history. In chapter 5 we consider another Sulawesi endemic, the Pale-blue Monarch (*Hypothymis puella*), along with its widespread relative the Island Monarch (*Monarcha cinerascens*). The Island Monarch was one of the original examples of a “supertramp species”, hypothesised to be a dispersal specialist excluded from larger islands by sedentary competitors like the Pale-blue Monarch. This chapter, however, argues for a slightly different interpretation, as I found that Island Monarch populations have diverged on even relatively small geographic scales, while the Pale-blue Monarch exhibits signs of gene flow across its range. The last of these core chapters (Chapter 6) presents a case that populations of two sunbird species, the Olive-backed Sunbird (*Cinnyris jugularis*) and the Black Sunbird (*Leptocoma aspasia*) exhibit such strong divergence that the species should be split. One of these populations (the “Wakatobi Sunbird *Cinnyris infrenatus*”, currently named as a subspecies) warrants recognition as an endemic species on the grounds of both genetic divergence and integrative taxonomy. The divergence of sunbird populations appears to be driven by both small islands, with their unique evolutionary conditions, and large biogeographic barriers inhibiting gene flow between regions.

Chapter 7, the final data chapter, offers a broad examination of gene flow between islands all over the world using previously published sequence data. Bird populations on pairs of islands are seen to be more genetically distinct from each other when their islands are separated by wider expanses of water. This chapter reinforces a conclusion suggested by all the preceding chapters: diversification of island birds is driven by both the geology of islands (producing barriers to gene flow) and the ecology and behaviour of birds (making some more likely to overcome the barriers than others). Knowledge of biodiversity, classic biogeographic theory, and modern methods of data collection and analysis are all required if we are to address the fundamental question: how species have been, and are being, evolved.

In memory of

my father

Fiachra Ó Marcaigh

and my friend

Aoibheann Gaughran

# Acknowledgements

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A biogeographer would be nothing without maps. The maps in this thesis were created thanks to the Natural Earth dataset, ArcGIS (thanks to TCD for the license), and the ESA's Copernicus imagery. Thanks in particular to BirdLife International for responding to my requests for IBA and species range data.

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This research would have been impossible without the Irish Research Council, who funded it under application (GOIPG/2017/1618), and Trinity's tradition of Foundation Scholarship. I will never forget the moments when these awards were announced.

Due to pandemic-related closures and the loss of my research space to construction work, much of this thesis was written without access to a university library. This was only possible thanks to people who have worked to provide free online access to the scientific literature, on websites such as the Biodiversity Heritage Library and the Internet Archive. Thanks also to SH, AE, and LG.

Lastly, I thank my friends and family for their invaluable support. There are too many names to list: if you think this might apply to you, then it does.

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The supplementary figures and tables (those with “S” in their number) are in the appendices in Volume II.

# Chapter 1 - General Introduction

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*“It is for such inquiries the modern naturalist collects his materials; it is for this that he still wants to add to the apparently boundless treasures of our national museums, and will never rest satisfied as long as the native country, the geographical distribution, and the amount of variation of any living thing remains imperfectly known. He looks upon every species of animal and plant now living as the individual letters which go to make up one of the volumes of our earth's history; and, as a few lost letters may make a sentence unintelligible, so the extinction of the numerous forms of life which the progress of cultivation invariably entails will necessarily render obscure this invaluable record of the past. ...*

*“If this is not done, future ages will certainly look back upon us as a people so immersed in the pursuit of wealth as to be blind to higher considerations. They will charge us with having culpably allowed the destruction of some of those records of Creation which we had it in our power to preserve; and while professing to regard every living thing as the direct handiwork and best evidence of a Creator, yet, with a strange inconsistency, seeing many of them perish irrecoverably from the face of the earth, uncared for and unknown.” – Alfred Russel Wallace (1863)*

**Chapter Illustration:** “My house at Bessir, in Waigiou” from Wallace (1869). Wallace is just visible underneath the house, working at a wicker table. “Waigiou” (now Waigeo) is an island off New Guinea.

## 1.1 Islands of life: the avian archipelago

It has long been appreciated by biologists that evolution can only be understood in the context of the physical and geological world in which it takes place. Darwin (1859) spent two chapters of his “Origin of Species” on the geological record, and a further two chapters on the geographical distribution of species. Wallace (1859, 1860, 1876, 1880, 1887, etc.) published a great body of work on the distribution of species across the world, which he recognised as a record of geological and evolutionary history. Both authors gained world-changing insights from their biogeographic studies, consisting of a close examination of the distribution of species with a particular focus on islands.

It is not hard to see why islands have played a key role in biogeography and evolutionary biology, when we consider two things. First, islands simply hold a striking and disproportionate amount of endemic biodiversity. Ten out of the 25 threatened biodiversity hotspots proposed by Myers *et al.* (2000) are made up largely or entirely of islands, including the Madagascar and Wallacea regions dealt with in this thesis. A revised version of this hotspot scheme added even more islands to the list (Whittaker and Fernández-Palacios 2007). Around 17-19% of all non-marine bird species are endemic to islands (Newton 2003, Tershy *et al.* 2015). Second, islands lend themselves well to study by biologists (Emerson 2002, Graham *et al.* 2017), as they are ecologically diverse and physically discrete, due to their being surrounded by water. These water barriers tend to impede the movement of organisms more effectively than other kinds of “barriers” around mainland habitats (Pujolar *et al.* 2022). These two factors have allowed biogeographers to use islands as “natural laboratories” in which to develop and test various theories (Whittaker *et al.* 2017).

Islands present different environments for evolution depending on their geological origins. Wallace (1880) divided the islands of the world into three biogeographic categories, based on both their geology and the organisms that occurred on them. In this thesis I refer to these categories using modern nomenclature (Whittaker and Fernández-Palacios 2007). Continental islands, also known as shelf or land-bridge islands, are those which sit on the continental shelf (Ali 2018). Most continental islands were connected to continents by land-bridges when sea levels were lower (Garg *et al.* 2022), and so are expected to hold biotas similar to those of the continents (Wallace 1887). Continental fragments are composed of separate pieces of continental crust, and so are effectively small continents in themselves. They are separated tectonically from the continents (Flantua *et al.* 2020), and are noted for particularly high levels of endemism (Whittaker and Fernández-Palacios 2007). Oceanic islands are produced by underwater volcanism or by coral reefs, and so

are physically isolated while being generally smaller, younger, and less stable than continental fragments (Wallace 1887). More recent work (Ali 2017, 2018) has made more detailed classifications based on a more developed understanding of geology. However, the aspects of islands that are most relevant to this thesis, particularly their size, stability, and degree of isolation, are captured adequately by the three categories outlined by Wallace (1880).

Islands have driven the evolution of life to such an extent that the nature and distribution of islands is reflected in that of species themselves. As land is scattered across the seas in a diverse array of discrete islands, each one shaped by the surrounding waters and by a complex geological history, so too is life (Wallace 1880). The plants, birds, and other terrestrial biotas that have evolved on islands, can thus be looked at as biogeographic “archipelagoes” in themselves. Of these, the “avian archipelago” has played a particular role in our understanding of how new species arise (Darwin 1859, Mayr and Diamond 2001) and is the main focus of this thesis.

## 1.2 What we don't know: Linnean and Wallacean shortfalls

Despite their importance, there is much we don't know about islands and the species inhabiting them. One goal of this thesis was to address some of these shortfalls, with a particular emphasis on Sulawesi and its surrounding islands in Wallacea. Worldwide, the number of species is so vast that it outmatches our ability to name them (Dubois 2010, Engel *et al.* 2021). This leads to a “taxonomic gap” (Raposo *et al.* 2021) or “Linnean shortfall” (Raven and Wilson 1992, Hortal *et al.* 2015), where we have not named many of the species which exist. When we address this Linnean shortfall by more accurately appraising species-level diversity, the result is an increase in endemism, as many of the newly identified species will be endemic to smaller areas (Nori *et al.* 2022). As they tend to be relatively restricted in their ranges, undescribed and newly described species are disproportionately likely to be at risk of extinction, and this pattern has been found to be particularly marked in birds (Liu *et al.* 2022). The result is that many species have gone extinct while still undescribed (Pimm *et al.* 2006, Lees and Pimm 2015), “uncared for and unknown” (Wallace 1863).

As well as a Linnean shortfall, biodiversity is subject to a Wallacean shortfall, i.e. a lack of information on species distributions (Lomolino 2004). Given the pivotal role that such distributions played in the development of evolutionary biology, this can only impede our understanding of how Earth's biodiversity has



evolved. Our imprecise knowledge of species distributions manifests in inaccurate maps used in conservation, many of which (especially in Southeast Asia) show an unlikely correspondence between species boundaries and political or administrative borders (Hughes *et al.* 2021). The avifauna of Sulawesi has been subject to severe Wallacean and Linnean shortfalls, but recent work, including some connected to this thesis, is improving the situation rapidly. To illustrate, Eaton *et al.* (2021) provide accounts of 27 additional endemic bird species and more precise range maps compared to the same book's first edition, published just over four years earlier. Despite its staggering endemism, higher than any other comparable land area on Earth (Safford and Hawkins 2013, Hawkins *et al.* 2015), the avifauna of Madagascar is still poorly known in several ways (Younger *et al.* 2018, Younger *et al.* 2019). During the course of this thesis, I contributed to several studies that helped to address Wallacean shortfalls in the Sulawesi and Madagascar regions (see Chapter 2 and the Additional Work section). Tackling Linnean shortfalls in Sulawesi and surrounding regions was a general goal of the three genetic sequencing chapters that form the core of this thesis. The data for these studies came from field expeditions to Sulawesi and Madagascar that took place over a number of years. The Sulawesi expeditions began in 1999 and ended in 2017 and were organised in collaboration between Trinity College Dublin, Universitas Halu Oleo in Kendari, and the conservation organisation Operation Wallacea. The Madagascar expeditions took place between 2010 and 2018 and were organised by Operation Wallacea, the Malagasy NGO "Development and Biodiversity Conservation Action for Madagascar" (DBCAM), and the University of Antananarivo. More detailed attributions and itineraries are presented at the beginning of each data chapter and in the respective Methods sections.

Wallacean and Linnean shortfalls are a significant issue for conservation, as assessment of extinction risk relies on knowledge of range sizes. Many taxa will go extinct before they are ever named (Whittaker *et al.* 2005). While other kinds of knowledge shortfall have been identified in evolution and ecology, the Wallacean and Linnean shortfalls are thought to have the most pervasive impact, even underlying and exacerbating the other kinds of knowledge shortfalls (Hortal *et al.* 2015). These shortfalls affect even relatively well-studied groups, like birds (Lohman *et al.* 2010, Lees *et al.* 2020), and ecosystems which we already know to be important, such as the Important Bird Areas (IBAs; Donald *et al.* 2019) and Endemic Bird Areas (EBAs; Stattersfield *et al.* 1998) designated by BirdLife International (Ó Marcaigh *et al.* 2020, O'Connell *et al.* 2020b). As well as their overall contribution to biodiversity, island species are particularly vulnerable to human disturbances such as habitat loss and invasive species (Graham *et al.* 2017). They are also disproportionately represented among endangered and extinct species (Tershy *et al.* 2015).

### 1.3 Sulawesi and Madagascar as continental fragments

Even among the world's islands, some stand out both for the rich endemism of their biotas and their importance to evolutionary biology. Wallace (1876) wrote of Sulawesi (or Celebes, as it was known in his time) that it was “in many respects the most remarkable and interesting [island] in the whole region, or perhaps on the globe, since no other island seems to present so many curious problems for solution.” Wallace (1880) was unsure how to fit Sulawesi into his classification scheme, considering it “anomalous”. As our understanding of Sulawesi's geology has improved greatly since then (Hall 2002, Satyana and Purwaningsih 2011, Hall 2012, 2013, Nugraha and Hall 2018), we now see that the island is a microcontinental terrane, or several continental fragments combined into one landmass (Michaux and Ung 2021). Sulawesi thus has the most in common with the “continental fragment” islands like Madagascar. Sulawesi is recognised as an Endemic Bird Area (Stattersfield *et al.* 1998), but due to its composition of multiple continental fragments there are several areas of endemism with distinct biotas (Evans *et al.* 2003, Trethowan *et al.* 2020, Ó Marcaigh *et al.* 2021b), and recent workers have emphasised that Sulawesi should be treated as more of a complex of several areas of endemism rather than a single geological unit (Ung *et al.* 2016, Michaux and Ung 2021).

Continental fragments are known for holding high numbers of endemic species. Older biogeographers (Wallace 1880) saw these islands as palaeo-endemic “museums” (Jablonski *et al.* 2006), meaning that they preserve taxa that have gone extinct in the rest of the world. Recent work has shown that much of the biodiversity of Sulawesi and Madagascar evolved after colonising across water (Yoder and Nowak 2006, Frantz *et al.* 2018), so these islands also function as neo-endemic “cradles” (Jablonski *et al.* 2006). The biotas of Sulawesi and Madagascar are considered “super-endemic” for their very high overall levels of endemism, comprising palaeo- and neo-endemic elements (Veron *et al.* 2019). This super-endemism is facilitated by the fact that continental fragments are relatively large, isolated, and old as landmasses (Flantua *et al.* 2020). Such high levels of endemism make Sulawesi and Madagascar urgent priorities for conservation: not only are so many of their species found nowhere else, but endemic species are more adversely affected by threats such as climate change (Manes *et al.* 2021).

Multiple processes contribute to the staggering endemic biodiversity seen on the continental fragments. Among these, dispersal is the movement of individuals leading to potential gene flow (Ronce 2007), as when birds fly between islands. Speciation is the evolutionary process that divides organisms into

discrete non-interbreeding arrays, or species (Dobzhansky 1940). This thesis investigates several questions about dispersal and speciation in Sulawesi, including identifying the islands and taxa most likely to generate new species and contribute to the biodiversity of this threatened hotspot. Other processes that influence population structure include extinction, range contraction, genetic bottlenecks, and hybridisation (Mayr 1963).

In addition to its own complex geology and high levels of endemism, Sulawesi is also of interest due to its unique position. Wallace (1863) identified the Makassar Strait to Sulawesi's west as part of a major biogeographic barrier, which came to be known as Wallace's Line (Huxley 1868). Heilprin (1887) and Lydekker (1896) described another such barrier west of New Guinea, now known as Lydekker's Line. These lines mark the boundaries of the shallow Sunda and Sahul continental shelves and the deeper waters between them (Van Welzen *et al.* 2011). As sea levels have changed during glaciations (Voris 2000), these boundaries have acted as barriers to the dispersal of many organisms, leading to marked differences in the plants and animals found on either side (Lohman *et al.* 2011). Later authors (Merrill 1924, Dickerson *et al.* 1928, Darlington 1957) saw Wallace's and Lydekker's Lines as the edges of a transition zone, and coined the name "Wallacea" for the region of deep water in between, over which this transition occurs. Wallacea has since been recognised as a threatened biodiversity hotspot (Myers *et al.* 2000) with a vital role in the evolution of songbirds, allowing them to disperse and radiate after originating in Australia (Moyle *et al.* 2016). The concepts of Wallace's Line, Lydekker's Line, and Wallacea have been applied in a somewhat confused way over the years: see Ali and Heaney (2021) for details and clarifications. The impacts of the deep water barriers represented by Wallace's and Lydekker's Lines are seen in the population structure described in Chapters 5 and 6 of this thesis.

## 1.4 Fragments of fragments: Sulawesi's satellite islands

As a microcontinental terrane, Sulawesi is composed of four peninsulae, called Northern, Central, South, and Southeast Sulawesi. In addition to the island of Sulawesi itself, the complex geology of this terrane has also formed a number of satellite islands off Sulawesi's coasts. These differ from each other in size and extent of isolation, making for an interesting natural laboratory to investigate biogeographic questions. Southeast Sulawesi sits on a fragment of continental lithosphere with the islands of Kabaena, Muna, and Wawonii (or Wowoni) (Hall 2013). The collision between this fragment and an adjoining microcontinent formed Buton (or Butung) Island (Satyana and Purwaningsih 2011). The seas between Sulawesi and these four islands are

narrow and shallow enough that they were connected by land within the last 20,000 years (Nugraha and Hall 2018), allowing them to function as continental islands in the sense of Wallace (1880). The Wakatobi Islands sit on the Tukang-Besi platform to the southeast, which is attached to the Buton microcontinental block, but may have collided with it after the block's collision with Southeast Sulawesi (Hall 2002, Satyana and Purwaningsih 2011). As the Wakatobi Islands are small, geologically young, and have always been separated by water barriers from Sulawesi and its land-bridge islands (Nugraha and Hall 2018), they function, biogeographically, as oceanic islands by Wallace's (1880) definition even though their geological origins differ from the "true" oceanic islands. The same is true of Menui (or Manui), a small island of uplifted coral limestone north of Wawonii (Monkhouse *et al.* 2018).

Very little was known about the biotas of the islands off Southeast Sulawesi until recently. They were subject to Wallacean shortfalls so severe that Menui, for example, was not even mentioned in the species accounts or labelled on the maps of the region's major ornithological reference works (White and Bruce 1986, Coates and Bishop 1997). This situation has improved in recent years, due in part to the larger research effort of which this thesis forms a part (Martin *et al.* 2012, Rheindt *et al.* 2014, O'Connell *et al.* 2017, Monkhouse *et al.* 2018, O'Connell *et al.* 2019d, O'Connell *et al.* 2020b).

The lack of knowledge of Sulawesi's offshore islands came with an attendant ignorance of the diversity of their faunas. Chapters 3, 4, and 5 of this thesis reveal substantial unrecognised diversity on the islands around Southeast Sulawesi, helping to address a Linnean shortfall by identifying populations that warrant revisions in taxonomy. Thus, as we gain a more accurate picture of diversity, we gain an appreciation of smaller areas of endemism (Nori *et al.* 2022). Sulawesi represents multiple biogeographic units (Ung *et al.* 2016, Michaux and Ung 2021) but is currently represented by a single BirdLife EBA. Madagascar, by way of contrast, contains seven EBAs (Stattersfield *et al.* 1998) despite its geological nature as a single continental block (Ali 2018). As many of the populations found to be of evolutionary and biogeographic interest in this thesis were not recorded until recently, due to Wallacean shortfalls, it raises the question: How many more interesting populations are we ignorant of? Our understanding of the evolution of life is reliant on knowing what's there.

## 1.5 Biogeographic theories from natural laboratories

As well as being worthy of study for their own sake and for conservation purposes, the “natural laboratories” (Whittaker *et al.* 2017) of the world’s islands have been pivotal to the development of the concepts underlying biogeography. Birds are particularly well-suited to biogeographic considerations as, despite the remaining Linnean and Wallacean shortfalls, we still know more about them than we do about other groups of organisms (Tobias *et al.* 2020). The study of island birds has contributed fundamental insights into the impacts of the geological environment on evolution (Wallace 1863), the relationships between species and their environments (MacArthur and Wilson 1967), and the evolution of new species through speciation (Mayr and Diamond 2001). The dawn of molecular phylogenetics has provided new ways for biogeographic theories to be tested (Emerson 2002). As well as contributing knowledge on poorly understood systems, this thesis used molecular techniques to test a number of influential biogeographic ideas from the pre-molecular era. Addressing Wallacean shortfalls aids in this, as many of the populations found to be pertinent to these questions had not been recorded in the literature until recently. This includes, for example, the avifaunas of Kabaena and Menui. Others were neglected for many years, as in the case of the Wakatobi Islands. This section introduces the biogeographic theories investigated, with more information given in the relevant chapters and in the General Discussion.

Island ecosystems and organisms differ from those on continents in important ways. Island biotas are shaped by founder effects (Sendell-Price *et al.* 2021) and by unique evolutionary environments, where natural selection often functions differently than on the adjacent mainland (Leroy *et al.* 2021). There are particular suites of evolutionary changes that occur on islands, known as the “island rule” (Benítez-López *et al.* 2021) or a broader “island syndrome” (Adler and Levins 1994). Differences in population density can drive increased sexual dimorphism in island populations, as the males and females diverge from one another in order to reduce competition between the sexes (MacArthur *et al.* 1972, O’Connell *et al.* 2019a). While this phenomenon is usually described on oceanic islands, Chapter 3 of this thesis deals with a rarer case in a continental island system.

As well as differences between mainland and island systems, different island types bring their own biogeographic considerations. Wallace (1880) outlined the key differences between oceanic and continental islands. One major difference is that oceanic islands are predicted to hold endemic species, while the biotas of continental islands are expected to be effectively identical to those of the neighbouring continents

(Wallace 1887). Chapters 3 and 4 challenge this expectation, finding that organisms on continental islands can differ from their neighbours in several important ways. Many influential biogeographic theories were developed primarily using oceanic islands (e.g. MacArthur and Wilson 1967) and recent work has demonstrated that they do not apply as well to continental islands (Garg *et al.* 2022). Continental islands have generally received less attention from biogeographers (Meiri 2017), but are still capable of providing interesting insights (Itescu *et al.* 2020).

In terms of their origins, continental islands hold a cohort of organisms when they first separate from their continents, while oceanic islands emerge from the sea devoid of terrestrial organisms (Wallace 1880). The formation of barriers between populations (as when sea levels rise and continental islands become separated) is termed vicariance, while oceanic islands must be colonised by the dispersal of organisms across existing water barriers. Debate over the relative importance of these two processes, vicariance and dispersal, exercised biogeographers for many years. While theories based on dispersal were at one time dismissed as “pseudo-explanations” (Croizat *et al.* 1974), in the last few years such theories have been “in the ascendancy” (Whittaker *et al.* 2017). Molecular techniques have shone light on the dispersal histories of populations, for example revealing that a species endemic to the Wakatobi Islands has its closest relatives in the distant Solomon Islands (O’Connell *et al.* 2019c). Now we know that dispersal plays a key role in biogeography generally, and in the super-endemic biota of Sulawesi in particular (Veron *et al.* 2019), it becomes important to assess differences in dispersal ability between organisms, as these may lead to distinct evolutionary and biogeographic patterns. Most of the chapters of this thesis address aspects of dispersal and the ways these affect speciation, with Chapter 7 providing a broad synthesis of this question.

Dispersal abilities are not necessarily fixed traits. Wilson (1959, 1961) contributed a key insight: that the dispersal ability of a species can change over time. This idea, called the taxon cycle, was controversial for a number of years but was championed by studies in the Caribbean (Ricklefs and Cox 1972, Ricklefs and Bermingham 1999, 2002). Like other theories based around dispersal, modern molecular studies have vindicated the idea of the taxon cycle (Jønsson *et al.* 2014, Pepke *et al.* 2019, Tobias *et al.* 2020, Kennedy *et al.* 2022). This idea plays a major role in Chapter 5 of this thesis and is also discussed in Chapter 6.

Some species are noted for a particularly strong tendency to disperse and colonise islands, even compared with their close relatives. Diamond (1974) coined the term “supertramp strategy” to describe the

lifestyle of certain birds which are widespread on small oceanic islands in Melanesia, but missing from larger islands and continental islands. Diamond hypothesised that these species represent dispersal specialists with poor competitive ability, a hypothesis expanded upon by Mayr and Diamond (2001). Recent molecular work (Linck *et al.* 2016, Pepke *et al.* 2019) has suggested that the supertramp strategy is not a permanent specialisation, but rather a transitional stage of the taxon cycle. Chapter 5 of this thesis applies similar methods to another of the “supertramp species” from Diamond (1974).

## 1.6 Thesis structure

In this thesis, I explore the diversity and speciation of island birds in several ways. There are six data chapters, each one dealing with a larger geographic area than the one before. My main aims were:

1. To help address some Linnean and Wallacean shortfalls in our knowledge of island birds, as any more complex theories rely on accurate knowledge of the biota itself.
2. To explore the population structure of birds in the important biodiversity hotspot of Sulawesi and its surrounding regions, supplying insights into evolutionary processes.
3. To use the patterns thus uncovered to refine certain concepts from biogeography, in particular our understanding of how differences in the dispersal ability of birds affect gene flow. This, in turn, drives patterns of speciation and thus the generation of biodiversity.

### **Chapter 2 – The avifauna of Ankobohobo Wetland, a neglected Important Bird Area in northwestern Madagascar**

**Study area:** a 35km<sup>2</sup> mangrove IBA in northwest Madagascar

Madagascar is the classic example of a continental fragment or “ancient continental” island (Wallace 1880). Like Sulawesi it is recognised for its “super-endemism” (Veron *et al.* 2019), and like the Wallacea region it is one of the originally defined biodiversity hotspots (Myers *et al.* 2000). Of all the biodiversity hotspots, Madagascar has been called one of the “hottest” (Ganzhorn *et al.* 2001). However, there are significant shortfalls in our knowledge of its highly threatened endemic avifauna (Younger *et al.* 2018, Younger *et al.* 2019), including Important Bird Areas that have not received proper ornithological attention (Palfrey *et al.* 2019). This chapter presents the first detailed species inventory of the Ankobohobo Wetland IBA, helping to

address Wallacean shortfalls of a number of species by providing more precise data on where they occur in this corner of the island. It also provides information on the status of the Critically Endangered Malagasy Fish Eagle *Haliaeetus vociferoides* (Des Murs 1845) in the site, a species of which there are only around 240 individuals worldwide (BirdLife International 2020d). This chapter has been published as Ó Marcaigh *et al.* (2020).

### **Chapter 3 – Cryptic sexual dimorphism reveals differing selection pressures on continental islands**

**Study area:** Southeast Sulawesi and its land-bridge islands

The Sulawesi Babbler *Pellorneum celebense* (Strickland 1849) is a skulking bird that occupies the forest understorey, and is endemic to Sulawesi and its land-bridge islands (Billerman *et al.* 2022). This makes it an interesting study species in several ways. Firstly, it gives an insight into the ecology of a bird adapted to a continental island setting dominated by vicariance. This study identified a pattern of sexual dimorphism in body size in the Sulawesi Babbler, with males being larger than females. It found that this dimorphism was stronger on the land-bridge islands than on the Sulawesi mainland. This is in keeping with predictions based on differences in population density between mainland and island systems (MacArthur *et al.* 1972). This phenomenon is usually described on oceanic islands, so this study provides a rare example from a continental island system. Such marked sexual dimorphism was not previously known to occur in the babbler family, another example of how shortfalls in our knowledge of natural history can obscure our understanding of evolution and ecology. This chapter was published as Ó Marcaigh *et al.* (2021a).

### **Chapter 4 - Evolution in the understorey: the Sulawesi Babbler *Pellorneum celebense* (Passeriformes: Pellorneidae) has diverged rapidly on land-bridge islands in the Wallacean biodiversity hotspot**

**Study area:** Sulawesi, its land-bridge islands, and Borneo

This chapter continues to examine the Sulawesi Babbler in greater detail. This time, its population structure is revealed using molecular phylogenetics and integrative taxonomic techniques. The study found considerable population structure in this bird, in keeping with the prediction that its understorey lifestyle limits dispersal and gene flow (Harris and Reed 2002). The current taxonomy of this species, which merges the Central and Southeast Sulawesi populations into a single subspecies (White and Bruce 1986, Gill *et al.* 2022), was not supported, as these populations were found to be evolutionarily independent from one another. This reflects Sulawesi's geological history, with the multiple microcontinental units as centres of endemism. Off the



Southeast Sulawesi coast, the Kabaena and Wawonii populations were found to be of particular interest, possibly due to the distinct ultramafic geology of their habitats (Galey *et al.* 2017). These two islands had not been surveyed ornithologically until recently (O’Connell *et al.* 2017, O’Connell *et al.* 2019d). This is an example of how the Wallacean shortfall limits our understanding, since we cannot study the structure of populations we do not know of. This chapter was published as Ó Marcaigh *et al.* (2021b).

### **Chapter 5 - Tramps in transition: genetic differentiation between populations of an iconic "supertramp" taxon in the Central Indo-Pacific**

**Study area:** Sulawesi, its land-bridge islands, Menui, the Wakatobi Islands, and Northern Melanesia

This chapter examines the population structure of the Island Monarch *Monarcha cinerascens* (Temminck 1827), which was one of the original examples of a “supertramp species” (Diamond 1974), and the related Sulawesi endemic Pale-blue Monarch *Hypothymis puella* (Wallace 1862). The study found strong population structure in the “supertramp” Island Monarch across the oceanic islands where it lives, including a potential species-level division between Wallacea and Melanesia, two regions on either side of Lydekker’s Line. The Menui population, which was not recorded until recently (Monkhouse *et al.* 2018), proves to be of biogeographic interest, evidence that Wallacean shortfalls can impede our knowledge of biogeography. This chapter supports recent papers (Linck *et al.* 2016, Pepke *et al.* 2019) that integrate the supertramp and taxon cycle hypotheses. This chapter has been published as Ó Marcaigh *et al.* (2022b).

### **Chapter 6 - Small islands and large biogeographic barriers have driven contrasting speciation patterns in Indo-Pacific sunbirds**

**Study area:** Sulawesi, its land-bridge islands, Menui, the Wakatobi Islands, Australia, New Guinea, the Solomon Islands, Borneo, and the Philippines

This chapter examines population structure in two sunbird species in Wallacea and neighbouring regions. The Olive-backed Sunbird *Cinnyris jugularis* (Linnaeus 1766, page 185) has been proposed to be split by recent authors (Eaton *et al.* 2021), but the Black Sunbird *Leptocoma aspasia* (Lesson and Garnot 1828) has not. The chapter finds that the diversity of both birds is underestimated by current taxonomy (Gill *et al.* 2022) and both are likely to merit species-level splits. This offers further proof that there are Linnean shortfalls still to be addressed in this super-endemic region, and reinforces the importance of Wallace’s Line and Lydekker’s Line as biogeographic barriers. The Wakatobi population of the Olive-backed Sunbird is proposed as an

endemic species, re-emphasising that the Sulawesi EBA comprises multiple centres of endemism, and the Wakatobi Islands should be recognised as a separate EBA. This chapter has been published as Ó Marcaigh *et al.* (2022a).

### **Chapter 7 - The genetic divergence between bird populations on islands is driven by both ecology and geology**

**Study area:** A total of 373 marine islands worldwide

The final data chapter uses a systematic review to bring together sequence data from 185 previously published papers, to test the relationship between dispersal ability and genetic divergence on the world's islands. This is a culmination of many ideas that feature in the other chapters, including differences between continental islands, oceanic islands, and continental fragments, and the effect of a bird's lifestyle and morphology on its dispersal and gene flow. A total of 716 species from around the world are included, allowing the project's biogeographic ideas to be tested on a broad scale. The chapter looks at pairs of birds on islands and measures the minimum distance between the islands as a measure of the water barrier separating the populations. A Bayesian modelling approach is then used to investigate how the genetic divergence across these barriers is affected by island type and by the foraging stratum and dispersal ability of the birds. We find that understory species and birds with wings ill-suited to long-distance dispersal are generally more genetically divergent across water barriers than their counterparts. This chapter uses previously published datasets (Wilman *et al.* 2014, Sheard *et al.* 2020), showing how natural history information can be synthesised into large-scale macroecological investigations. After the final acceptance of this thesis, this chapter will be developed into a final manuscript and submitted to a biogeographic journal.

### **Chapter 8 – Discussion**

In the final chapter of my thesis, I draw general conclusions and outline how the preceding data chapters have advanced the thesis aims, as well as recommending future avenues of research.

## 1.7 Additional work

In addition to the data chapters enclosed in this thesis, my PhD provided me the opportunity to contribute to several other research projects. I assisted with several studies which provided species inventories for sites in Sulawesi and Madagascar, and one that identified two new species of *Zosterops* white-eyes in the Wakatobi Islands. Thus, each of these papers helped to address Wallacean or Linnean shortfalls in super-endemic regions. They were published as follows:

- O'Connell, D. P., Sealy, S., **Ó Marcaigh, F.**, Karya, A., Bahrún, A., Analuddin, K., Kelly, D. J., and Marples, N. M. (2017). The avifauna of Kabaena Island, south-east Sulawesi, Indonesia. *Forktail* **33**, 40-45.
- O'Connell, D. P., **Ó Marcaigh, F.**, O'Neill, A., Griffin, R., Karya, A., Analuddin, K., Kelly, D. J., and Marples, N. M. (2019). The avifauna of Muna and Wawonii Island, with additional records from mainland South-east Sulawesi, Indonesia. *Forktail* **35**, 50-56.
- O'Connell, D. P., Kelly, D. J., Lawless, N., O'Brien, K., **Ó Marcaigh, F.**, Karya, A., Analuddin, K., and Marples, N. M. (2019). A sympatric pair of undescribed white-eye species (Aves: Zosteropidae: *Zosterops*) with different origins. *Zoological Journal of the Linnean Society* **186**, 701-724. doi:10.1093/zoolinnean/zlz022
- Palfrey, R. H., Baddams, J., Raveloson, B. A., Rasamison, S., **Ó Marcaigh, F.**, Neaves, J., Long, P., and Martin, T. E. (2019). The avifauna of the forest mosaic habitats of the Mariarano region, Mahajanga II district, north-west Madagascar. *Bothalia* **49**, a2416.

## Chapter 2 - The avifauna of Ankobohobo Wetland, a neglected Important Bird Area in northwest Madagascar

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*“The corporeal bird, the feathered bird,  
who forces a tunnel through the wind  
to get to the moon he’s seen in a dream  
among the branches  
falls with the night  
into a labyrinth of leaves.”*

- Jean-Joseph Rabearivelo (1934)

**Author Contribution:** I conceived this study with Thomas E. Martin and we led the writing of the manuscript. Peter Long designed the field methodologies and curated the data set. I collected the field data with Bruno Andriandraotomalaza Raveloson, Gael Rakotomanga, Anja Navalona Ratianarivo, Jack Baddams, Solohery Rasamison, and Jamie Neaves. I created Figure 2.1, the other figures are photographs and are attributed in their captions.

**Status:** This paper has been published Open Access in *Scopus: Journal of East African Ornithology*, the journal of the Bird Committee of the East Africa Natural History Society. The journal version is available at <https://bit.ly/3wBd2Ln>

**Citation:** Ó Marcaigh, F., Raveloson, B. A., Rakotomanga, G., Ratianarivo, A. N., Baddams, J., Rasamison, S., Neaves, J., Long, P., and Martin, T. E. (2020). The avifauna of Ankobohobo Wetland, a neglected Important Bird Area in northwestern Madagascar. *Scopus: Journal of East African Ornithology* **40**, 18-28.

**Chapter Illustration:** Malagasy Fish Eagle, from Des Murs (1849).

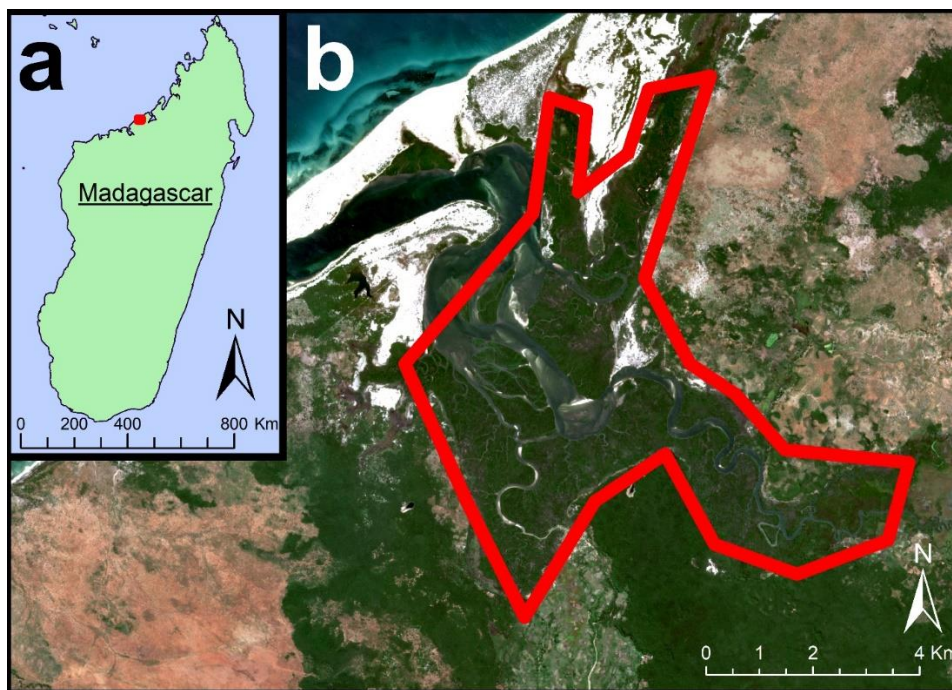
## 2.1 Summary

We present here the first detailed inventory of the birds of Ankobohobo Wetland in northwest Madagascar, based on data collected annually in June and July 2010-2018. These wetlands consist of a c. 35 km<sup>2</sup> area of mangroves and tidal mudflats which were designated as an Important Bird Area (IBA) within the West Malagasy Wetlands Endemic Bird Area (EBA) in 2001. However, recent and detailed information on their avifauna is lacking. We used a boat to survey three 4km stretches of the IBA's river system on four repeated occasions each year, supplemented by opportunistic observations made in various parts of the study area. In total, we detected 59 species in Ankobohobo Wetland through c. 608 h of observation effort. This includes 26 Malagasy endemics, two Near Threatened species (Curlew Sandpiper *Calidris ferruginea* and Lesser Flamingo *Phoeniconaias minor*), three Endangered species (Malagasy Sacred Ibis *Threskiornis bernieri*, Malagasy Pond Heron *Ardeola idae*, and Humblot's Heron *Ardea humbloti*), and the Critically Endangered Malagasy Fish Eagle *Haliaeetus vociferoides*. These constitute substantial additions to the inventory of the established Ankobohobo Wetland IBA, which previously stood at 19 species including one Malagasy endemic. We summarise these records here, providing additional details for threatened species. We also report observed threats to the wetlands, particularly with regards to the breeding *H. vociferoides* population, and highlight Ankobohobo as an important conservation priority.

## 2.2 Introduction

Madagascar possesses one of the most irreplaceable yet threatened biotas on Earth (Myers *et al.* 2000, Goodman and Benstead 2005), which for centuries has been a source of fascination for both the international scientific community (e.g. de Flacourt 1658, Grandidier 1885) and oral tradition and local heritage (Burney and Ramilisonina 1999, Jones *et al.* 2008). Over half (53%) of the island's terrestrial breeding birds are endemic (Warren *et al.* 2013), many of which are now threatened with extinction. These threats have become so great as to prompt concerns that the coming years may represent the "last chance" to safeguard the country's unique natural heritage (Jones *et al.* 2019). A key first step towards safeguarding biodiversity is to identify priority areas in which to focus conservation resources (Brooks *et al.* 2006). BirdLife International's Important Bird and Biodiversity Area (IBA) programme is a highly successful example, shown to drive tangible conservation outcomes (Donald *et al.* 2019, Waliczky *et al.* 2019).

There are 84 IBAs in Madagascar, but several of these have not had follow-up surveys since they were designated and lack complete species inventories. This includes Ankobohobo Wetland (IBA MG022, BirdLife International 2020b), a relatively small IBA encompassing 34.97 km<sup>2</sup> of mangrove, tidal mudflats, and some sandy beaches. The site is located 80km northeast of Mahajanga city in the Boeny region of northwestern Madagascar (Figure 2.1). Ankobohobo sits between two larger coastal wetland IBAs, Baie de Bombetoka (MG024, 55km to Ankobohobo’s southwest) and the Mahajamba Bay-Anjavavy Complex (MG023, 32km to the northeast), within the West Malagasy Wetlands Endemic Bird Area (EBA) (ZICOMA 2001). This EBA has been highlighted as being of urgent priority and incomplete knowledge (Stattersfield *et al.* 1998, BirdLife International 2020a). Ankobohobo’s IBA designation was based on surveys carried out in 1997, reporting populations of two threatened and biome-restricted species (Endangered Humblot’s Heron *Ardea humbloti* and Critically Endangered Malagasy Fish Eagle *Haliaeetus vociferoides*), along with 17 others (BirdLife International 2020b). Other than this, no further information on the site has been published apart from two species-specific reviews of the status of *H. vociferoides* which include data from the IBA (Rabarisoa *et al.* 1997, Razafimanjato *et al.* 2014).



**Figure 2.1.** a) Map of Madagascar with the Ankobohobo area marked in red. b) Satellite photo of the study area, with the boundary of the Ankobohobo Wetland IBA shown in red. Created in ArcMap (ESRI 2020) using Copernicus Sentinel-2 imagery (ESA 2020), with IBA boundary data provided by BirdLife International (2020b).

The lack of data from the Ankobohobo IBA represents an important knowledge gap, as the conservation situation in Ankobohobo, as in Madagascar generally, has deteriorated considerably in the two decades since the site was first gazetted. A disproportionate number of Madagascar's threatened endemic birds rely on wetland ecosystems (Young *et al.* 2014), but Madagascar's mangroves are increasingly threatened by demand for charcoal and *sokay*, a type of lime used to strengthen houses that requires mangrove wood and seashells to produce (Scales *et al.* 2018). Protection of Madagascar's important sites thus depends on further research into how its species are distributed. Palfrey *et al.* (2019) recorded numerous previously undocumented species in the Mariarano forest region adjacent to Ankobohobo, but noted that Ankobohobo would benefit from more intensive exploration. We therefore present here the most detailed information published to date on the avifauna of this globally significant IBA.

## 2.3 Methods

The birds of Ankobohobo Wetland were surveyed between 2010 and 2018, as part of a long-term ecological monitoring programme run in partnership between Operation Wallacea, the Malagasy NGO 'Development and Biodiversity Conservation Action for Madagascar' (DBCAM), the University of Antananarivo, and local community forest management groups. I participated in the 2018 field season, and conceived with Thomas E. Martin to use the species list from the monitoring dataset for an avifaunal inventory paper. Surveys took place in the dry season over periods of 6–7 weeks between June and August, through semi-structured boat surveys. We conducted these boat surveys on three stretches of the IBA's river system, each 4km long, each on four repeated occasions per year. These surveys involved recording every bird seen or heard, while travelling in one direction along a set route. We also made incidental opportunistic records in the course of completing ecological research into the local Nile Crocodile *Crocodylus niloticus* population, and through other casual exploration of the study area.

Using records from all our surveys and opportunistic observations, we compiled a full inventory of all species we had detected in Ankobohobo Wetland, following the taxonomy of Gill *et al.* (2020). We obtained the global conservation status of each species from the IUCN (2020), and noted where a species was endemic to the Madagascar biodiversity hotspot as defined by Myers *et al.* (2000). The IUCN system divides threatened species into three categories of increasing risk: Vulnerable, Endangered, and Critically Endangered. We checked maps provided in authoritative online resources (BirdLife International and NatureServe 2014, del Hoyo *et al.* 2020, IUCN 2020) and three of the region's widely-used ornithological

references (Safford and Hawkins 2013, Sinclair and Langrand 2013, Hawkins *et al.* 2015) to see if the species we had recorded were indicated as occurring in the study area. We also assigned categorical abundance estimates for each species in our inventory based on frequency of observations, following Palfrey *et al.* (2019). Abundant species were those typically recorded several times each day in suitable habitat; common species were typically recorded once per day, fairly common species were typically recorded about once per week, uncommon species had an average of fewer than five or six records per field season, and rare species were known from fewer than five observations within the study area. We also noted the author who observed each species. Finally, as recommended by Lees *et al.* (2014), we collated all the photographs we had taken of study species within the study area, and uploaded them to the “Internet Bird Collection” online depository (Lynx Edicions 2020), which has since become part of the Macaulay Library (Cornell Lab of Ornithology 2020). These photos are available at <https://www.macaulaylibrary.org/> using the catalog numbers in Table 2.1, providing visual verifications for as many species in our inventory as possible.

## 2.4 Results

We recorded 59 species in Ankobohobo Wetland, including 26 endemic species (44% of all species detected), two Near Threatened species, three Endangered species, and one Critically Endangered species. We obtained photographic records for 27 species in our inventory, and a sound recording for one additional species for which we could not obtain a photograph (Malagasy Swamp Warbler *Acrocephalus newtoni*). Table 2.1 summarises our findings. The following accounts provide further details on notable records such as endemic and threatened species.

**Table 2.1.** Checklist of bird species recorded in Ankobohobo Wetland between 2010 and 2018. All taxonomy follows Gill *et al.* (2020). Species marked \* are endemic to the Madagascar biodiversity hotspot as defined by Myers *et al.* (2000). Species marked † are assessed as threatened or near threatened by the IUCN (2020). Species marked (I) are introduced to the study area. Abundance estimates are denoted as follows: A = abundant; C = common; Fc = fairly common; U = uncommon; R = rare. Initials in the ‘observers’ column indicate authors possessing records of each species. Species which have been observed by three or more authors are notated ‘multiple’. FÓM is Fionn Ó Marcaigh, BAR is Bruno Andriandraotomalaza Raveloson, JN is Jamie Neaves, and JB is Jack Baddams. Catalog Numbers correspond to photographs and sound recordings from this study available on the Macaulay Library (Cornell Lab of Ornithology 2020).

Family	Common name	Scientific name	Abundance	Observers	Catalog No.
Anatidae	White-faced Whistling Duck	<i>Dendrocygna viduata</i>	R	FÓM	



	Knob-billed Duck	<i>Sarkidiornis melanotos</i>	R	FÓM	
<b>Phoenicopteridae</b>	Lesser Flamingo†	<i>Phoeniconaias minor</i>	R	BAR	
<b>Phalacrocoracidae</b>	Reed Cormorant	<i>Microcarbo africanus</i>	U	BAR, JN	ML712311
<b>Ciconiidae</b>	African Openbill	<i>Anastomus lamelligerus</i>	R	BAR	ML712312
<b>Threskiornithidae</b>	Malagasy Sacred Ibis*†	<i>Threskiornis bernieri</i>	R	BAR	ML712310
	African Spoonbill	<i>Platalea alba</i>	U	BAR, JN	ML712305
<b>Ardeidae</b>	Little Bittern	<i>Ixobrychus minutus</i>	R	BAR, JN	
	Black-crowned Night Heron	<i>Nycticorax nycticorax</i>	Fc	Multiple	ML204695631
	Striated Heron	<i>Butorides striata</i>	Fc	Multiple	ML204678131
	Squacco Heron	<i>Ardeola ralloides</i>	U	BAR, JN	
	Malagasy Pond Heron*†	<i>Ardeola idae</i>	R	JN	ML712380
	Grey Heron	<i>Ardea cinerea</i>	Fc	BAR, JN	ML712379
	Humblot's Heron*†	<i>Ardea humbloti</i>	R	BAR	
	Purple Heron	<i>Ardea purpurea</i>	Fc	Multiple	ML204678081
	Great Egret	<i>Ardea alba</i>	Fc	Multiple	ML713014
	Black Heron	<i>Egretta ardesiaca</i>	U	BAR, JB	ML204695731
	Dimorphic Egret*	<i>Egretta dimorpha</i>	C	Multiple	ML204695781
<b>Anhingidae</b>	African Darter	<i>Anhinga rufa</i>	U	Multiple	ML204695601
<b>Accipitridae</b>	Malagasy Harrier-Hawk*	<i>Polyboroides radiatus</i>	R	JB	ML204678271
	Black Kite	<i>Milvus migrans</i>	R	FÓM	ML713003
	Malagasy Fish Eagle*†	<i>Haliaeetus vociferoides</i>	U	Multiple	ML712309
	Malagasy Buzzard*	<i>Buteo brachypterus</i>	R	FÓM	ML713004
	Bat Hawk	<i>Macheiramphus alcinus</i>	R	JN	
<b>Rallidae</b>	White-throated Rail*	<i>Dryolimnas cuvieri</i>	C	Multiple	ML204678251
	Common Moorhen	<i>Gallinula chloropus</i>	Fc	FÓM	ML713009
<b>Dromadidae</b>	Crab-plover	<i>Dromas ardeola</i>	R	BAR	
<b>Charadriidae</b>	Common Ringed Plover	<i>Charadrius hiaticula</i>	U	BAR	
	White-fronted Plover	<i>Charadrius marginatus</i>	U	BAR	
<b>Scolopacidae</b>	Eurasian Whimbrel	<i>Numenius phaeopus</i>	Fc	BAR, JN	
	Curlew Sandpiper†	<i>Calidris ferruginea</i>	Fc	BAR	
	Sanderling	<i>Calidris alba</i>	U	BAR	
	Terek Sandpiper	<i>Xenus cinereus</i>	Fc	BAR	
	Common Sandpiper	<i>Actitis hypoleucos</i>	Fc	Multiple	ML204695741
	Common Greenshank	<i>Tringa nebularia</i>	Fc	BAR, JN	
<b>Laridae</b>	Lesser Crested Tern	<i>Thalasseus bengalensis</i>	Fc	BAR	
<b>Columbidae</b>	Malagasy Turtle Dove*	<i>Nesoenas picturatus</i>	R	FÓM	
	Namaqua Dove	<i>Oena capensis</i>	R	FÓM	ML713015
<b>Cuculidae</b>	Malagasy Coucal*	<i>Centropus toulou</i>	R	FÓM	
<b>Tytonidae</b>	Western Barn Owl	<i>Tyto alba</i>	R	JN	
<b>Caprimulgidae</b>	Malagasy Nightjar*	<i>Caprimulgus madagascariensis</i>	Fc	JN	
<b>Apodidae</b>	Malagasy Black Swift*	<i>Apus balstoni</i>	R	FÓM	

<b>Alcedinidae</b>	Malagasy Kingfisher*	<i>Corythornis vintsioides</i>	C	Multiple	ML713010
<b>Meropidae</b>	Olive Bee-eater	<i>Merops superciliosus</i>	C	JN, FÓM	ML713012
<b>Falconidae</b>	Malagasy Kestrel*	<i>Falco newtoni</i>	R	FÓM	ML713008
<b>Psittacidae</b>	Lesser Vasa Parrot*	<i>Coracopsis nigra</i>	Fc	JN, FÓM	ML713013
	Grey-headed Lovebird*	<i>Agapornis canus</i>	R	FÓM	
<b>Vangidae</b>	White-headed Vanga*	<i>Artamella viridis</i>	R	FÓM	
<b>Dicruridae</b>	Crested Drongo*	<i>Dicrurus forficatus</i>	C	JN, FÓM	ML713011
<b>Monarchidae</b>	Malagasy Paradise Flycatcher*	<i>Terpsiphone mutata</i>	C	JN, FÓM	
<b>Corvidae</b>	Pied Crow	<i>Corvus albus</i>	R	FÓM	ML713007
<b>Pycnonotidae</b>	Malagasy Bulbul*	<i>Hypsipetes madagascariensis</i>	R	FÓM	
<b>Acrocephalidae</b>	Malagasy Swamp Warbler*	<i>Acrocephalus newtoni</i>	Fc	BAR, JB	ML203945641
<b>Cisticolidae</b>	Common Jery*	<i>Neomixis tenella</i>	R	FÓM	
<b>Sturnidae</b>	Common Myna (I)	<i>Acridotheres tristis</i>	R	FÓM	
	Malagasy Starling*	<i>Hartlaubius auratus</i>	R	FÓM	
<b>Nectariniidae</b>	Souimanga Sunbird*	<i>Cinnyris sovimanga</i>	R	FÓM	
<b>Estrildidae</b>	Madagascan Mannikin*	<i>Lepidopygia nana</i>	U	FÓM	
<b>Motacillidae</b>	Malagasy Wagtail*	<i>Motacilla flaviventris</i>	Fc	JN, FÓM	

### **MALAGASY SACRED IBIS *Threskiornis bernieri* - Endangered**

A rare resident. Singles and pairs were observed several times by BAR on sandbanks alongside mangrove-fringed channels in 2011 and 2013 (Figure 2.2). The presence of this species here is not unexpected, given known distributions suggest a theoretical occurrence in suitable habitat anywhere on the western coast of Madagascar, and particularly as this section of coast corresponds to the core part of its range (Safford and Hawkins 2013). However, it has not been explicitly reported from Ankobohobo Wetland previously, being absent from the IBA summary for this site (BirdLife International 2020b).

This species has experienced a rapid population decline of over 20% in the last 16 years. The current global population estimate is 1500-1850 birds, which is expected to decline further due to harvesting of eggs, disturbance of nesting sites, and the degradation of wetland habitats in Madagascar (BirdLife International 2020e). Ankobohobo represents an important portion of the wetland habitat that must be protected if this species is to be conserved.



**Figure 2.2.** Malagasy Sacred Ibis *Threskiornis bernieri* by Bruno Andriandraotomalaza Raveloson.

#### **MALAGASY POND HERON *Ardeola idae* - Endangered**

A rare migrant. We have occasionally observed single individuals on sandbanks along mangrove-fringed channels (Figure 2.3). The species has a widespread but small population throughout Madagascar, where it breeds in the austral summer between October and March (BirdLife International 2020c). The population of this species has declined substantially in recent decades due to habitat destruction and exploitation at breeding sites (Rabarisoa *et al.* 2020), and the global population is now estimated at just 1100 breeding birds. The species winters on the mainland of East Africa between May and September, but our photograph (ML712380) provides rare documentation of an individual remaining in the breeding range during that time. Wintering records of the species in the adjacent Mariarano forest region have also been reported (Palfrey *et al.* 2019). Rabarisoa *et al.* (2020) recorded 911 instances of this species remaining in Madagascar during the austral winter, but a search of literature and eBird records revealed few other photographs documenting birds in winter plumage in Madagascar. Rabarisoa *et al.* (2020) found western habitats to be particularly important for this species, highlighting the need to protect sites such as Ankobohobo.



**Figure 2.3.** Malagasy Pond Heron *Ardeola idae* by Jamie Neaves.

**HUMBLLOT'S HERON** *Ardea humbloti* - Endangered

A rare resident. Single individuals have occasionally been observed by BAR on sandbanks of mangrove-fringed channels and on coastal beaches. The presence of this species in Ankobohobo has been reported previously (BirdLife International 2020b).

**LESSER FLAMINGO** *Phoeniconaias minor* – Near Threatened

A rare visitor to the wetland. BAR observed two individuals in 2011 on a coastal beach on the fringes of Ankobohobo.

**CURLEW SANDPIPER** *Calidris ferruginea* – Near Threatened

A locally common non-breeding population occupies the same area of coastal beach where the Lesser Flamingo was observed. As in the Malagasy Pond Heron, breeding individuals leave Madagascar in March-April and return in September-October, but some non-breeding individuals remain all year (Hawkins *et al.* 2015). This area was last surveyed (by BAR) in 2011.

**MALAGASY STARLING** *Hartlaubius auratus* – Least Concern

A rare visitor to the wetland. A small flock was observed by FÓM above the wetland's main river system in 2018. Usually a bird of forests and shrublands (Safford and Hawkins 2013), its presence in Ankobohobo likely results from the proximity to the Mariarano forest, where it was recorded by Palfrey *et al.* (2019).

**MALAGASY FISH EAGLE** *Haliaeetus vociferoides* - Critically Endangered

An uncommon resident. Occasionally recorded within the study area (Figure 2.4), where a few pairs have been known to nest in tall mangrove trees (BirdLife International 2020b). The maximum number of individuals we observed at a single time was three birds: a breeding pair and one chick. An image providing evidence of breeding can be found in our photographic inventory (ML712417, Figure 2.5). The species was observed breeding at this location annually between 2010 and 2017. Individuals have also occasionally been seen passing over the wetlands of the adjoining Mariarano forest, but they have not been observed to breed there (Palfrey *et al.* 2019). Our observations show that the *H. vociferoides* population in Ankobohobo, while small, is certainly larger than indicated by the last two reviews of the status of this species. Rabarisoa *et al.* (1997) reported only a single bird in Ankobohobo, while Razafimanjato *et al.* (2014) did not represent the species as still persisting here at all. We expect the population size here to be broadly in line with that of the initial IBA assessment of 2-3 breeding pairs (BirdLife International 2020b). There are only an estimated 240 individuals of this species remaining globally (BirdLife International 2020d), marking this Ankobohobo population as significant. However, this population may now be under severe threat. In 2013 the nesting trees where the eagles consistently bred showed signs of human damage, although whether this was due to firewood collection or a deliberate act of persecution remains unclear. As a matter of greater concern, a visit to Ankobohobo on 25 June 2018 by JN revealed that these nesting trees had been completely destroyed, and that deforestation for charcoal production was much more apparent in the area than in previous survey seasons. The 2018 survey season was the first year since 2010 where no breeding birds were recorded, though sub-adult and adult individuals were still observed. These recent disturbances raise serious concerns regarding the future conservation status of the species here.



**Figure 2.4.** Malagasy Fish Eagle *Haliaeetus vociferoides* by Jamie Neaves.

**Figure 2.5.** Breeding *H. vociferoides* by Jamie Neaves.

## 2.5 Discussion

Globally, wetlands are crucial for biodiversity and for humanity, but their protection requires effective governance and better data (Amano *et al.* 2018). Mangroves in particular provide enormous ecosystem services, but globally they are being lost at an accelerating rate (Polidoro *et al.* 2010). Our surveys in the mangroves of Ankobohobo Wetland have yielded valuable records that boost the known biological value of this IBA. Where previous surveys had recorded 19 species, our records add a further 40, including 25 more endemic species and five that are Threatened or Near-Threatened, thus greatly improving knowledge of the site. The 59 species and 44% endemism rate reported here compare with 95 species and a 66.3% endemism rate in the adjacent Mariarano forest landscape (Palfrey *et al.* 2019). Lower diversity and endemism are to be expected in Ankobohobo given that it is a smaller and more homogenous area, and a wetland rather than a forest, but our results still highlight its diversity as being of regional importance. Aside from the presence of endemic and globally threatened species here, this relatively small habitat fragment has been shown to support a significant proportion of the region's bird species, as the 59 species reported here represent over a third (35%) of the 168 non-vagrant species known to occur in western Madagascar (Safford and Hawkins 2013). If the boundaries of the Ankobohobo IBA were extended to encompass the Mariarano forest, then this larger IBA would contain substantially more threatened and range-restricted bird species and greater habitat diversity, and would still be viable for conservation as one unit due to its well-defined and relatively small area. Sites have been similarly combined into complexes elsewhere in western Madagascar, including in the neighbouring Mahajamba Bay – Anjavavy Complex. In addition to its birds, Ankobohobo supports populations

of other threatened taxa, for example a roost of approximately 500 Madagascar Flying Fox *Pteropus rufus*, which is considered Vulnerable by the IUCN (2020). The site is also utilized by troops of the Endangered Coquerel's Sifaka *Propithecus coquereli*. Thus, if the two sites were combined as one Ankobohobo-Mariarano Complex, this might warrant additional designation, perhaps under the IUCN's Key Biodiversity Area scheme. The long-term monitoring scheme that gave rise to this study could provide data to assess such proposals. Alternatively, as Mariarano and Ankobohobo represent quite different ecosystems, with each supporting different IBA/KBA trigger species, they are also capable of meeting the criteria for these designations independently. Two IBAs in different habitats within a small area would emphasise this region's ecological richness and diversity. Therefore, we recommend that one or other of these approaches be followed, recognising the international importance of both Ankobohobo and Mariarano so that legal protection may follow.

Our results have highlighted that the Ankobohobo IBA faces severe environmental pressures. The most significant of these concerns the destruction of *H. vociferoides* nesting sites, but general deforestation (particularly associated with charcoal burning) has been frequently observed here, particularly around the area's periphery. Urgent conservation actions are therefore needed to safeguard the future of the site, and it is likely that the IBA designation alone is not sufficient to provide meaningful protection. Conservation interventions such as community education to highlight the importance of the *H. vociferoides* population, and provision of alternate means of fuel to mitigate deforestation, are recommended.

Decades after the designation of the Ankobohobo Wetland IBA, and centuries after the rich natural heritage of Madagascar began to be formally described, detailed species inventories are still needed for the adequate understanding and protection of the truly remarkable Malagasy wetlands. Such shortfalls in knowledge are a continuing issue with respect to nature conservation in Madagascar and around the world (de Lima *et al.* 2011, Pino-Del-Carpio *et al.* 2014). Malagasy poet Jean-Joseph Rabearivelo used an image of a bird "falling with the night" (Rabearivelo 1934), which may reflect the future of Madagascar's birds without adequate knowledge and protection.

## Chapter 3 - Cryptic sexual dimorphism reveals differing selection pressures on continental islands

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*"Then certain philosophers of the Epicureans, and of the Stoicks, encountered him.  
And some said, What will this babbler say?" - Acts 17:18*

**Author Contribution:** I conceived this study with David J. Kelly and Nicola Marples, and we wrote the manuscript together. David Kelly and Nicola Marples led the field work, with contributions from Adi Karya, Kangkuso Analuddin, and me. I carried out the lab work (with Naomi Lawless) and the morphometric analyses. I produced all of the figures. David and Analuddin produced a second abstract in Bahasa Indonesia, available in Appendix S3.1.

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**Chapter Illustration:** Sulawesi Babbler, from the original species description by Strickland (1849).



## 3.1 Summary

Birds are well known for their sexual dimorphism. But not all forms of dimorphism are the same, and differences in morphology can be so subtle that they aren't detected by casual observation. We report that this is the case with the Sulawesi Babbler (*Pellorneum celebense*), the first reported instance of sexual dimorphism in this species or any of the ground babblers of the Southeast Asian islands. Our finding is based on a combination of morphometric analyses, genetic sexing, and observation of breeding condition. We highlight the utility of unsupervised clustering approaches, widely used in the biomedical literature, for the investigation of sexual dimorphism in ecological and evolutionary contexts. The sexual dimorphism was weaker on the mainland of Sulawesi and stronger on the continental islands of Kabaena, Muna, and Buton. This suggests that different evolutionary pressures have led the species to partition niches differently in these habitats, which separated only recently in geological history. This kind of intraspecific niche partitioning is an intrinsic part of the ecological niche of such species, one we must not miss if we are to fully understand these endlessly fascinating organisms and systems. A summary in Bahasa Indonesia is available in Appendix S3.1.

## 3.2 Introduction

From the Bee Hummingbird (Berns and Adams 2012) to the Ostrich (Mine *et al.* 2002), sexual dimorphism is a common feature of bird species. Different bird species display different forms of dimorphism, including sex-based differences in size, plumage colour, social and sexual behaviour, and parental care (Owens and Hartley 1998). Traditionally, differences between species in the extent of sexual dimorphism were attributed to their mating and parental care systems (Darwin 1871). More recent work has suggested different evolutionary mechanisms behind body-size and plumage-colour dimorphisms, with size dimorphism explained largely by intrasexual competition (Owens and Hartley 1998).

Each form of sexual dimorphism comes with its own ecological and evolutionary implications. For instance, sexual dimorphism in plumage colour (dichromatism) has been used as an index of the strength of sexual selection in a species (Phillimore *et al.* 2006), and thus linked to the diversification of clades (Barraclough *et al.* 1995) and to extinction risk following island introductions (McLain *et al.* 1999). Studies based on descriptions in field guides found that the majority of passerine birds (69%) are monochromatic, and do not exhibit differences in plumage colour between sexes (Barraclough *et al.* 1995). However, differences in the colour perception abilities of humans and birds suggest many examples of dichromatism

may have been missed (Eaton 2005). As other forms of dimorphism may be more subtle than dichromatism, they are even more likely to have been overlooked.

Sexual dimorphism in size is associated with sexual selection through territory size and mate acquisition, and with natural selection through ecological divergence (Krüger *et al.* 2007). Differences in morphology between males and females enable them to utilise niches differently (Selander 1966), and so reduce intersexual competition (González-Solís *et al.* 2000). This is especially important in island systems, where birds can sometimes occur at higher densities due to reduced interspecific competition (MacArthur *et al.* 1972), causing intraspecific competition to be stronger than on larger landmasses (Robinson-Wolrath and Owens 2003). At the same time, island populations may be released from interspecies competition that they experience in more species-rich mainland settings, allowing them to expand into vacant niches (Diamond 1970).

The Sulawesi Babbler (*Pellorneum celebense*) is a member of the Pellorneidae, or ground babblers, a bird family recently created to accommodate species separated from the Timaliidae, Sylviidae, and Cisticolidae on molecular evidence (Cai *et al.* 2019). It has taken scientists many years to untangle the complex systematics of this group (Cibois *et al.* 2002, Cibois 2003, Gelang *et al.* 2009, Moyle *et al.* 2012, Cai *et al.* 2019), and many aspects of their natural history remain enigmatic. Endemic Sulawesi Babbler subspecies have been described from the north and southwest peninsulae of Sulawesi and the small island of Togian (respectively *P. c. celebense*, *finschi*, and *togianense*), while the southeast peninsula and its islands are considered to share the *P. c. rufofuscum* subspecies with central Sulawesi (Billerman *et al.* 2022). The Sulawesi Babbler has been described as “shy” and “relatively featureless”, and accounts of other pellorneid babblers have highlighted a lack of information on their breeding habits, diet, and voice (Billerman *et al.* 2022). Due to their cryptic appearance and shy behaviour of skulking in the forest understorey, birds like babblers tend to be less well understood than other groups, to the point that entire species may remain undiscovered (Gaston and Blackburn 1994). The only pellorneid babblers previously described as sexually dimorphic are the grass-babblers of the Indian subcontinent, while the phrase “sexes similar” is frequently seen in accounts of babblers in *Pellorneum* and other Southeast Asian genera (Billerman *et al.* 2022). Though its plumage is dull and not sexually dichromatic, the Sulawesi Babbler is known to sing in duet, a relatively rare trait generally found in territorial birds with stable social bonds (Tobias *et al.* 2016).

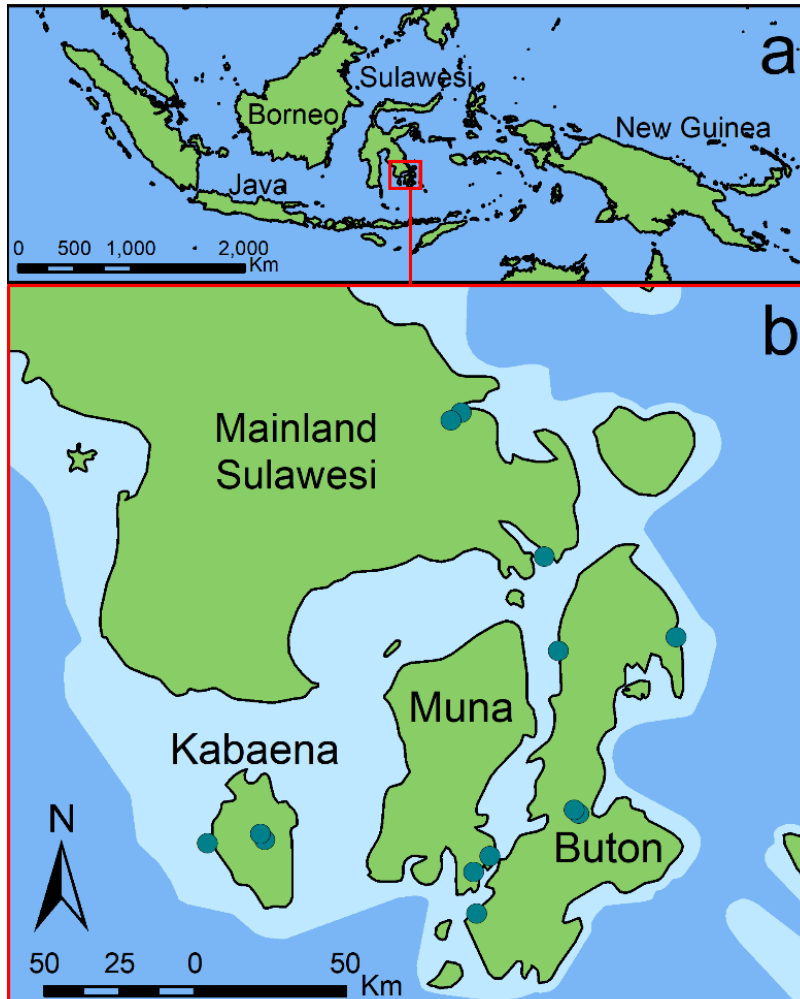
Just like their vocalisations, any differences in morphology between male and female birds can inform us as to their ecological relationships and the evolutionary conditions that have shaped them. This is particularly important in places like Sulawesi, a treasure trove of endemic species (Rheindt *et al.* 2020) in one of the world's threatened biodiversity hotspots (Myers *et al.* 2000). So complex both geologically and biologically that Wallace (1880) deemed it "anomalous", Sulawesi has been of interest to evolutionary biologists throughout the history of the field (e.g. Mayr and Vaurie 1948, MacArthur and Wilson 1963), and a full understanding of endemic species like the Sulawesi Babbler can only add to this foundational store of knowledge. The southeast peninsula of Sulawesi (Figure 3.1) shares its sliver of continental lithosphere with the islands of Kabaena and Muna (Hall 2013), while Buton Island formed through collision with an adjoining microcontinent (Satyana and Purwaningsih 2011). All four islands are separated by narrow and shallow seas, and were connected by land within the last 20,000 years (Nugraha and Hall 2018). Thus they function as "continental" islands in the biogeographic sense of Wallace (1880, 1887) despite the absence of a true continent (Ali 2018). As there has been so little time for their populations to evolve in isolation, anything that sets the populations of Kabaena, Muna, and Buton apart from those of Sulawesi must have evolved at a rapid pace (Rheindt *et al.* 2020). We have investigated the evolution of the Sulawesi Babbler on these continental islands, in the process observing bimodal distributions in morphometrics which prompted questions about sexual dimorphism in this ostensibly monomorphic species. This study aims to clarify the nature of this dimorphism and its geographic underpinnings, building on what is known about these cryptic tropical birds and this biodiverse region while also providing an example of the role of sexual dimorphism in ecology and evolution.

## 3.3 Methods

### 3.3.1 Sample and data collection

Birds were sampled by mist netting during expeditions to southeast Sulawesi between 1999 and 2017 (O'Connell *et al.* 2019c). We captured babblers from the *P. c. rufofuscum* populations on the southeast peninsula of Sulawesi, and on the smaller islands of Kabaena, Muna, and Buton (Figure 3.1, Table S3.1). Captured birds were measured using the methods recommended by Redfern and Clark (2001) to obtain morphometric data including wing length (maximum chord), bill length (tip of bill to the base), skull length (from back of skull to base of bill), weight, and tarsus length (minimum). Only adult birds measured by NMM were included in morphometric analyses. Breeding condition was assessed as described in Svensson (1992). Most of these birds were also sampled for the genetic analysis: a small number of contour feathers were

taken from the flank of each bird and stored in sealed paper envelopes. Sampling of contour feathers rather than any other feather tracts minimises the risk of injury to the bird and avoids disrupting its flight ability and any plumage-based visual signals (McDonald and Griffith 2011).



**Figure 3.1.**

a) Map of the Indonesian archipelago with our sampling region outlined in red.

b) Map of Southeast Sulawesi. Sampling sites are marked with blue circles. Waters coloured in a lighter blue are less than 200m in depth, the darker seas are deeper than 200m. All maps were produced using ArcGIS (ESRI 2020).

### 3.3.2 Unsupervised clustering

All morphometric analyses were carried out in R version 3.5.2 (R Core Team 2021). The data used are available in the supplementary materials (Table S3.2). Having observed apparent bimodal patterns in some of

our data (Figure S3.1), we used expectation–maximization (EM) functions in the R package *mixtools* (Benaglia *et al.* 2009) to investigate these patterns further, identifying which traits could be sorted into two overlapping normal distributions so that these could be retained for our unsupervised clustering analysis.

To investigate the morphological patterns in more detail, we performed cluster analysis using the R package *mclust* (Scrucca *et al.* 2016). The *mclust* algorithm uses the Bayesian Information Criterion (BIC) to select between clustering models, and to make an unbiased calculation of the number of mixture components (or “clusters”) in the data. This approach is known as unsupervised clustering, and it allows one to discern and describe subgroups of individuals even when the subgroups cannot be readily identified by eye (Benaglia *et al.* 2009). Unsupervised clustering is thus well suited for detecting body size dimorphism in a monochromatic bird like the Sulawesi Babbler. Unsupervised clustering has been used to examine sexual dimorphism of mice and humans in the biomedical literature (e.g. Basant *et al.* 2010, El Wakil *et al.* 2013, Mwangi *et al.* 2014), but appears to be used less often in ecology and evolution.

### 3.3.3 Genetic sexing

In parallel to our morphometric investigations, we investigated sexual dimorphism as a possible explanation for the patterns they revealed. During sampling in the field, we were able to sex some of our birds based on their breeding condition, where breeding males exhibited cloacal protuberances and breeding females had brood patches. However, like many tropical birds the Sulawesi Babbler has a long breeding season, stretching from April to December (Billerman *et al.* 2022), so only a minority of our birds were breeding at the time we caught them. Therefore, we performed genetic sexing techniques on the tissue samples we had taken to identify males from females.

In birds sex is determined by the Z and W chromosomes, with homogametic (ZZ) individuals developing into males and heterogametic (ZW) individuals into females (Irwin 2018). Thus, molecular sexing techniques for birds involve detection of the Z and W chromosomes, usually by PCR amplification of the highly conserved chromobox-helicase-DNA-binding gene (CHD). The technique introduced by Griffiths *et al.* (1998) uses a single primer pair that bind to homologous sections of the Z-linked and W-linked versions of this gene (CHD1Z and CHD1W), amplifying across an intron. The section amplified is around 300-400bp in length, but as the DNA in the intron is noncoding, it varies in length between CHD1Z and CHD1W and between different species. Thus, when the PCR product is examined on an electrophoresis gel, male birds will

show single bands corresponding to CHD1Z, while female birds will display two bands of different lengths, for CHD1Z and CHD1W.

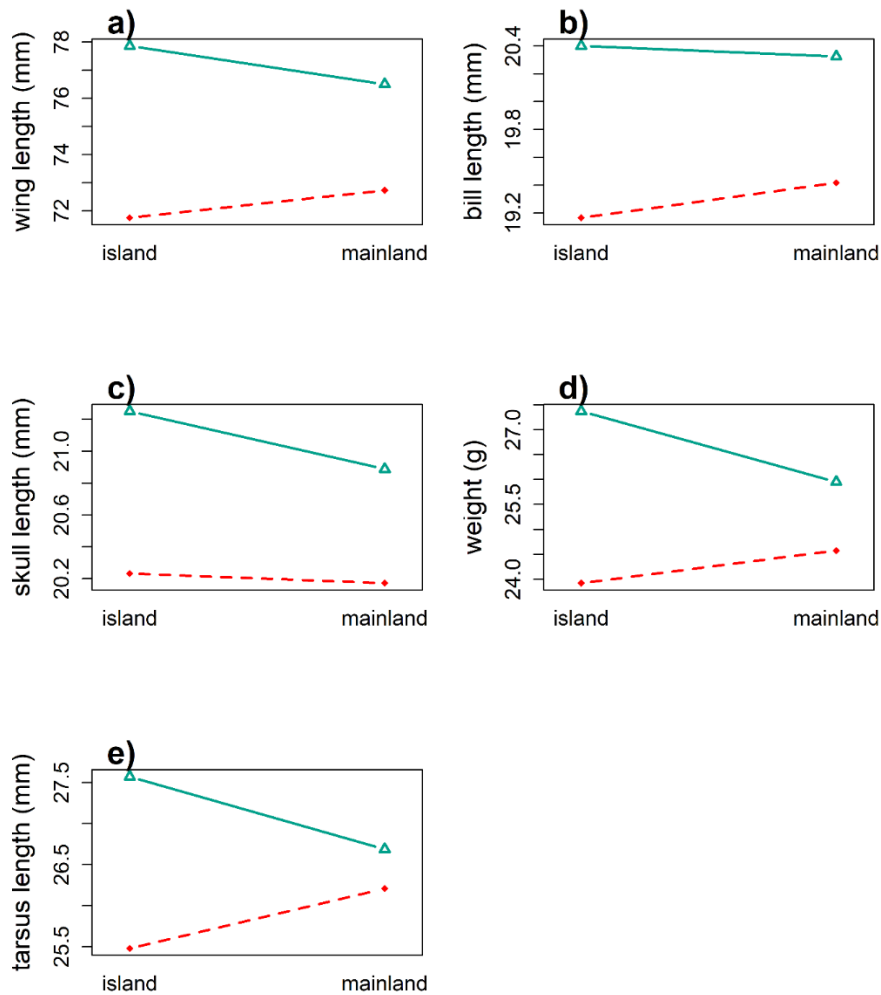
We carried out separate PCR reactions using the original P2 and P8 primers (each of these primers is 20bp long; Griffiths *et al.* 1998), and the CHD1-F/CHD1-R set (Lee *et al.* 2010), which are 23bp each and have performed better than P2/P8 in previous comparative analyses (Çakmak *et al.* 2017). The CHD1-F and CHD1-R primers target a different section of the CHD gene and their products are more variable in length than those of P2 and P8, with Lee *et al.* (2010) reporting products up to 800bp in some species. The sequences of all primers used are available in Table S3.3. Our 20 µl PCR reactions comprised 7.5 µl template DNA, 5.5 µl double-distilled water, 0.4 µl 10 mM deoxynucleoside triphosphates (dNTPs), 2 µl 10× PCR reaction buffer, 2.4 µl 25 mM MgCl<sub>2</sub>, 1 µl 10 µM forward primer, 1 µl 10 µM reverse primer, and 0.2 µl Taq polymerase. Figure S3.2 shows an example PCR protocol, including cycling program. These reactions were screened on 2% agarose gels to check for single and double bands.

### 3.3.4 Analysis of dimorphism

To investigate differences between mainland and island populations in sexual dimorphism, we expressed sexual dimorphism as the percentage difference of male to female morphology (Selander 1966, Santiago-Alarcon and Parker 2007, Greenberg and Danner 2013), using the following formula:

$$\text{Dimorphism} = ( (\text{mean male trait} / \text{mean female trait}) - 1 ) * 100$$

We designated birds as “male” and “female” based on the results of our unsupervised clustering analysis, as it was supported by the genetic sexing. We grouped the Kabaena, Buton, and Muna populations as “island” babblers, for comparison against “mainland” babblers from Sulawesi. We drew interaction plots to explore the relationship between the mclust-based “sex” categorisations and island or mainland population (Figure 3.2).



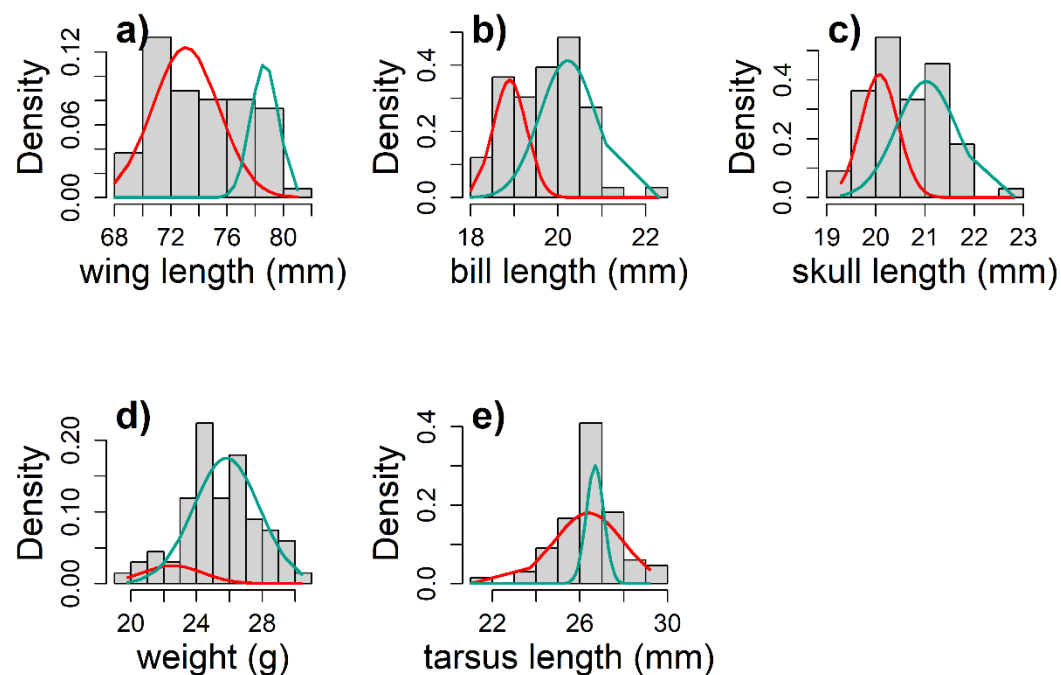
**Figure 3.2.** Interaction plots showing differences in sexual dimorphism between mainland (Sulawesi) and island (Kabaena, Muna, and Buton) populations, in our five morphometric variables a) wing length, b) bill length, c) skull length, d) tarsus length, and e) weight. Male birds are shown as blue triangles, females as red diamonds. The lines are not parallel, indicating an interaction between population and sex.

To test the patterns suggested by the interaction plots and dimorphism percentages, we ran a two-way Multivariate Analysis of Variance (MANOVA) using wing length, bill length, skull length, tarsus, and weight as the dependent variables. MANOVA assumes that the dependent variables are linearly related, but not overly correlated. Scatterplots of all five variables showed a linear relationship (Figure S3.3) and Pearson's correlation coefficients of these data were between 0.4 and 0.65 (Table S3.4), so these

assumptions were not violated. We also assessed normality of residuals using a histogram (Figure S3.3), to test a general assumption of Analysis of Variance.

### 3.4 Results

Our wing length, bill length, and skull length data showed strong bimodality and could be sorted by the EM Functions into two overlapping normal distributions (Figure 3.3). We therefore retained them for use in our unsupervised clustering analysis. Cluster analysis of these three traits identified two groups of birds across the island and mainland populations (Table 3.1). Based on the BIC, mclust selected an EEI (Equal Equal Isotropic) model with two mixture components, meaning that there were two diagonal “clusters” in the data, of equal volume and shape. Group 1 contained the 35 smaller birds, Group 2 the 31 larger ones (Figure 3.4).

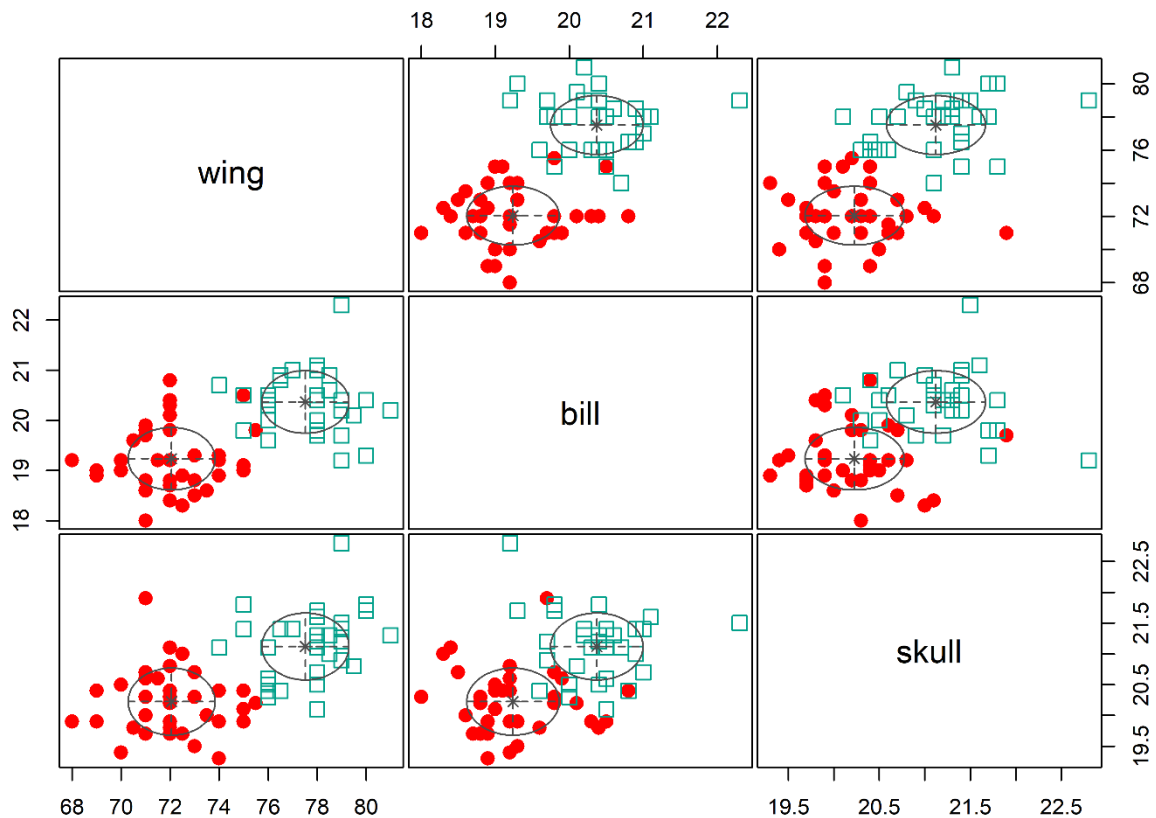


**Figure 3.3.** Histograms of babler morphometric data: **a)** wing length, **b)** bill length, **c)** skull length, **d)** weight, and **e)** tarsus length. The red and blue lines represent two overlapping normal distributions identified by expectation–maximization (EM) functions.



**Table 3.1.** Results of the unsupervised clustering analysis, with the number of birds sorted into each group on each island.

Group	1 (Female)	2 (Male)
Buton	11	11
Kabaena	7	11
Muna	6	0
Sulawesi	11	9



**Figure 3.4.** Plot of the mclust unsupervised clustering analysis on our babbler morphometric data. Two clusters were identified. Birds in Group 2 (larger individuals) are shown as blue squares, while Group 1 (smaller individuals) is represented by red circles. The ellipses represent the covariances of the mixture components, demonstrating that the morphological characters vary similarly in the two “clusters”.

The CHD1F and CHD1R primers failed to amplify many of our samples. In the seven samples where they did produce bands, they were single bands with molecular weight corresponding to CHD1W, indicating female birds. The P2 and P8 primers were more successful as they amplified 29 of our samples, producing the expected pattern whereby some birds had single bands (for male) and others had double bands (indicating females). This result sexed Group 2 birds as male and Group 1 birds as female in every case (Table 3.2), confirming that these morphological groupings had their basis in sex and that male babblers were larger than females. Where possible, we compared the results of the genetic sexing techniques and the visual assessment of breeding condition. The sex assigned to each individual on breeding condition was in accordance with the genetic sexing in every case (Table 3.2). This indicates that inspection of breeding condition, genetic sexing using the CHD gene, and morphometric sexing using unsupervised clustering were all valid and compatible methods.

**Table 3.2.** Summary of sexing results. Four sexing methods were employed, but not all could be applied to all birds. This table gives the number of birds (n) from each island sexed using each combination of methods. Breeding sex is based on breeding condition of birds in the hand. Morphological sex is based on our unsupervised clustering analysis, which sorted smaller birds (females) into Group 1 and larger birds (males) into Group 2. P2/P8 and CHD1F/CHD1R are two sets of PCR primers for genetic sexing, used to detect the presence of the sex chromosomes Z and W, where male birds have a ZZ karyotype and females ZW. Individual results for all birds used in this study are in Table S3.6.

Island	Breeding	Morphology	P2/P8	CHD1F/CHD1R	n
BUTON	M	Group 2			3
BUTON	F	Group 1			1
BUTON		Group 1	ZW		2
BUTON		Group 2	ZZ		2
BUTON		Group 1	ZW	W	1
KABAENA	M	Group 2			2
KABAENA	M	Group 2	ZZ		1
KABAENA		Group 1	ZW		5
KABAENA		Group 2	ZZ		7
KABAENA		Group 1	ZW	W	1
KABAENA			ZW	W	1
MUNA	F	Group 1			1
MUNA	F	Group 1		W	2
MUNA		Group 1		W	1
SULAWESI	F	Group 1			3
SULAWESI	M	Group 2			5
SULAWESI	F	Group 1	ZW		1
SULAWESI	F	Group 1	ZW	W	1
SULAWESI		Group 2	ZZ		2

<b>SULAWESI</b>	Group 1	ZW	1
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Island birds appeared more strongly dimorphic than their mainland counterparts in each of our five morphological traits, as shown in percentage dimorphism and interaction plots (Table 3.3 & Figure 3.2). This effect was particularly strong in tarsus length and weight. The two-way MANOVA on all five traits found a statistically significant interaction effect between population and sex ( $p < 0.05$ ), demonstrating that the island babblers showed a greater degree of sexual dimorphism than the mainland birds. The full output of this test is available in Table S3.5.

**Table 3.3.** Percentage of sexual dimorphism in morphometric traits of babblers from “Mainland” (Sulawesi)

<b>Measurement</b>	<b>% Dimorphism –</b>	
	<b>Mainland</b>	<b>Islands</b>
<b>Wing Length</b>	5.72	8.77
<b>Bill Length</b>	5.23	6.59
<b>Skull Length</b>	3.37	5.02
<b>Tarsus Length</b>	2.48	8.22
<b>Weight</b>	6.78	15.31

and “Island” (Kabaena, Buton, and Muna) populations. This sexual dimorphism metric is the ratio of mean “male” morphology to mean “female” morphology, centred on zero and expressed as a percentage.

### 3.5 Discussion

Our analyses are the first to demonstrate sexual dimorphism in any of the ground babblers of Southeast Asia. Collar and Robson (2007) wrote of another babbler family, the Timaliidae, that “differences between the sexes of babblers are mainly morphometric, with females almost invariably a little smaller than males, although this is only an average and there is plenty of overlap.” While this is consistent with the direction of the dimorphism we found, the magnitude appears to differ, as our male babblers were decidedly larger (Table 3.3, Figure 3.2) and showed little overlap with females (Figure 3.4).

Size dimorphism is harder to detect than other forms of sexual dimorphism, such as dichromatism, and can change our understanding of the evolution of morphology. As size dimorphism is now known from both pectoral and timaliid babblers, we recommend that similar analyses be carried out on other babbler species and on related taxa. Among the babblers' relatives are the white-eyes (Zosteropidae) (Cai *et al.* 2019), a taxon that has been pivotal to studies in evolutionary biology and biogeography for decades. Analysis of white-eye morphology has contributed to our understanding of speciation (O'Connell *et al.* 2019c), song evolution (Potvin 2013), and dispersal (Linck *et al.* 2016). If body-size dimorphism is found in members of this family, it could unlock further insights. Some genetic sexing work has been undertaken on white-eye species in the past (Frentiu *et al.* 2003), but such work must be linked to analyses of morphology if sexual dimorphism in size is to be identified. Museum collections could help to extend investigation of sexual dimorphism across a broad range of babblers and related species, providing access to both morphometrics and genetic material. Limitations to such a study would include the age of museum material, which may make amplification of the CHD gene more difficult, and the lack of head measurements due to the practice of removing the back of the skull during the preparation of bird specimens (Winker 2000).

The morphometric traits used in this study correspond to different aspects of the birds' ecology: wing length has a role in flight (Nowakowski *et al.* 2014) and bill length in diet (Pigot *et al.* 2020), while skull length corresponds to overall body size (Rising and Somers 1989). The patterns we have identified in these traits are therefore indicative of ecological differences between males and females, and between island and mainland systems. The island-mainland difference may relate to the concept of "density compensation", whereby species can occur at higher densities on islands with fewer interspecific competitors (MacArthur *et al.* 1972), leading to increased intraspecific competition (Robinson-Wolrath and Owens 2003). This, in turn, can drive increased sexual dimorphism compared to mainland populations. O'Connell *et al.* (2019a) found that Olive-backed Sunbirds (*Cinnyris jugularis*) occurred at significantly higher densities on Kabaena than on mainland Sulawesi. As we have found babblers to be more sexually dimorphic on Kabaena and the other islands than on the Sulawesi mainland, it may be the case that increased densities are having a similar effect in this species. Interestingly, O'Connell *et al.* (2019a) found that sunbirds on Buton occurred at a similar density to those on the mainland, and that those on the oceanic Wakatobi islands were more sexually dimorphic than those on both the Sulawesi mainland and the continental islands of Buton, Muna, and Kabaena, which they grouped together as the "mainland zone". The Sulawesi Babbler may thus be unusual in this region for exhibiting a diagnosable increase in sexual dimorphism on the continental islands of Kabaena, Muna, and Buton, which were connected to Sulawesi (and one another) by land bridges in recent geological history (Nugraha and Hall 2018). Understorey or skulking species like ground babblers are prone to speciate

on either side of river barriers (Smith *et al.* 2014), so a similar dispersal effect could be driving their evolutionary divergence on these islands.

Although birds are among the most well studied animals, there remains much to discover about their ecology and evolution, particularly in the tropics. The Sulawesi Babbler, being of a shy and skulking habit, cryptically coloured, and part of a complex phylogenetic radiation, represents one of the gaps in our understanding of both the familiar animals that are birds and the evolutionary hotspot that is Sulawesi. Moreover, the Sulawesi Babbler is one of Sulawesi's many endemic species (O'Connell *et al.* 2017), and the cryptic sexual dimorphism we have documented represents another form of hidden diversity in this beleaguered biodiversity hotspot (Myers *et al.* 2000). Finally, the fact that the babblers are more strongly dimorphic in a continental island setting has interesting implications for their ecology, suggesting that different pressures are exerted on male and female birds in different environments, and highlighting the fact that even recently formed islands can present unique evolutionary settings. More than a century after the passing of Alfred Russel Wallace, there remains much to learn about his "anomalous island" (Wallace 1880).

## Chapter 4 - Evolution in the understory: the Sulawesi Babbler *Pellorneum celebense* (Passeriformes: Pellorneidae) has diverged rapidly on land-bridge islands in the Wallacean biodiversity hotspot

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“... I once found a list of diseases as yet unclassified by medical science, and among these there occurred the word *Islomania*, which was described as a rare but by no means unknown affliction of spirit. There are people, Gideon used to say, by way of explanation, who find islands somehow irresistible. The mere knowledge that they are on an island, a little world surrounded by the sea, fills them with an indescribable intoxication.”

Lawrence Durrell (1953)



**Author Contribution:** I conceived this study and wrote the manuscript with Nicola Marples and David Kelly. Nicola and David led the field work, with contributions from Darren O’Connell, Kangkuso Analuddin, Adi Karya, and me. I carried out the labwork, with help from Naomi Lawless, Darren O’Connell, Daniel Dunleavy, and Alice Clark. I processed the song recordings and DNA sequences with Daniel Dunleavy. I analysed the data, with input from Darren on phylogenetics. I created all of the figures.

**Status:** This chapter was published Open Access in *Zoologischer Anzeiger – A Journal of Comparative Zoology*. The journal version is available at <https://bit.ly/3wledbT>

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**Chapter Illustration:** The *celebense* (bottom-left) and *finschi* (top-right) subspecies of Sulawesi Babbler, from Walden (1876).

## 4.1 Summary

Tropical islands hold great treasures of Earth's biodiversity, but these fragile ecosystems may be lost before their diversity is fully catalogued or the evolutionary processes that birthed it are understood. We ran comparative analyses on the ND2 and ND3 mitochondrial genes of the Sulawesi Babbler *Pellorneum celebense*, an understorey bird endemic to Sulawesi and its continental islands, along with its morphology and song. Genetic, acoustic, and morphological data agree on the recognition of multiple isolated populations. The Sulawesi Babbler shows signs of rapid speciation, with populations diverging between Central and Southeast Sulawesi, and even on land-bridge islands which were connected within the last few tens of thousands of years. The genetic divergence between Sulawesi Babbler populations in this time has been around 33% of their divergence from sister species which have been isolated from Sulawesi for millions of years. This is likely facilitated by the Sulawesi Babbler's understorey lifestyle, which inhibits gene flow and promotes speciation. Similar patterns of endemism are seen in Sulawesi's mammals and amphibians. This work highlights the undocumented biodiversity of a threatened hotspot, wrought by complex processes of speciation which interact with ecology and geology. Subspecific taxonomy has at times been controversial, but we argue that discrete populations such as these play a key role in evolution. Lying as they do at the heart of the biodiversity hotspot of Wallacea, these islands can reveal much about the evolution of biodiversity at all of its levels, from the gene to the ecosystem.

## 4.2 Introduction

The 20<sup>th</sup> century Biological Species Concept cemented the importance of isolation in evolutionary biology, with pivotal works like those of Mayr (1942, 1959) and Dobzhansky (1937, 1940) showing that populations become species when they are isolated, first geographically and then reproductively. The role of geographic isolation in speciation continues to inspire debate, particularly in the evolutionary marvels that are the world's islands (e.g. Flantua *et al.* 2020, Itescu *et al.* 2020). Early biogeographers such as Wallace (1880) noted that islands vary in their geographical isolation, with important consequences for evolution. Some, which they termed oceanic islands, were created by volcanic eruptions or uplift of coral far out at sea. Oceanic islands are separated from continents and other large landmasses by deep seas, and thus have never been connected to them by land. Continental land-bridge islands, on the other hand, are formed from parts of the continental shelf, and so at times of reduced sea level they were a continuous part of the mainland. Though later work has elaborated on this original classification (Ali 2017, 2018), much research on island speciation still focuses on highly isolated oceanic islands such as Hawaii and the Galápagos (Whittaker *et al.* 2017). A smaller, but growing, body of literature has drawn important evolutionary conclusions from the

faunas of land-bridge islands (e.g. Lister 1989, Vartanyan *et al.* 1993, Keogh *et al.* 2005, Itescu *et al.* 2020), which are more numerous, ecologically more complex, and zoologically richer than are oceanic islands (Meiri 2017). As land-bridge islands have been isolated only briefly, these studies provide evidence of evolution which has taken place at a rapid rate.

Isolation of an island population results from features of the species as well as the island, as some organisms are more likely to maintain gene flow across water barriers than others. Thus, studies of evolution on land-bridge islands tend to focus on particularly weak dispersers, such as terrestrial mammals and reptiles (e.g. Lister 1989, Vartanyan *et al.* 1993, Keogh *et al.* 2005). However, even among birds and other strongly dispersing animals, certain ecological and behavioural traits will inhibit gene flow across barriers which the organism should be physically capable of crossing (Harris and Reed 2002). Some birds that fly long distances over land will not cross even narrow bodies of water (Diamond 1981), and even the dispersive species that do colonise remote islands can develop “behavioural flightlessness” in these isolated populations (Moyle *et al.* 2009). The habit of foraging in forest understorey seems to be particularly significant in limiting gene flow and driving speciation (Burney and Brumfield 2009, Smith *et al.* 2014). This chapter aimed to investigate evolution on the land-bridge islands surrounding Sulawesi using a bird which is limited to the forest understorey, the endemic Sulawesi Babbler *Pellorneum celebense*. This species was first described, as *Trichastoma celebense*, by Strickland (1849). The genus *Trichastoma* (Blyth 1842) was subsumed into *Pellorneum* (Swainson 1831) by Moyle *et al.* (2012) and Cai *et al.* (2019). Our taxonomy follows Gill *et al.* (2022).

Sulawesi is the largest island of the Wallacea region, one of Earth’s threatened biodiversity hotspots (Myers *et al.* 2000). Sulawesi’s complex geology has shaped an “anomalous” biogeography (Wallace 1880) and a high level of endemism (Stattersfield *et al.* 1998), with the island divided into four distinct peninsulae or “arms”, here referred to as North, Central, South, and Southeast Sulawesi (Figure 4.1) (the centre of the island, between the arms, also forms part of Central Sulawesi). Southeast Sulawesi and the islands of Kabaena, Muna, and Wawonii (or Wowoni) all sit on a small fragment of continental lithosphere (Hall 2013), which collided with an adjoining microcontinent to form Buton (or Butung) Island (Satyana and Purwaningsih 2011). The seas between these five islands are both shallow and narrow, and they were connected by land within the last 20,000 years (Nugraha and Hall 2018).



The diversity of islands and species found in Sulawesi make it an ideal place to study evolutionary divergence across islands. As well as the land-bridge islands described here, there are more isolated islands, including the Sula group and the Wakatobi (or Tukangbesi) archipelago, which have never been connected to Sulawesi by land (Nugraha and Hall, 2018). Descriptions of endemic species from these more isolated islands indicate that birds readily speciate when separated by such permanent barriers (Kelly *et al.* 2014, O’Connell *et al.* 2019c, Rheindt *et al.* 2020). The Sulawesi Babbler is found on all the land-bridge islands of Southeast Sulawesi, making it an ideal candidate for studies of evolutionary divergence on this shorter time scale. As the populations on Sulawesi, Buton, Kabaena, Muna, and Wawonii have been separated for an evolutionarily brief period of time, any divergence between them is evidence of evolution occurring at a rapid pace. These islands remain poorly known ornithologically, with species inventories emerging only recently (Martin *et al.* 2012, Martin *et al.* 2015, Martin *et al.* 2017, O’Connell *et al.* 2017, O’Connell *et al.* 2019d). As pressures on Indonesia’s birds continue to mount (Rentschlar *et al.* 2018), it becomes increasingly urgent that we study recently documented populations such as these, in order to estimate their evolutionary distinctiveness and consider their conservation.

The Sulawesi Babbler belongs to the family Pellorneidae (ground babblers), which was formed after species were split from the Timaliidae, Sylviidae, and Cisticolidae on molecular evidence (Cai *et al.* 2019). Babbler systematics are complex, and scientists’ interpretations of them have changed repeatedly over the years (Cibois *et al.* 2002, Cibois 2003, Gelang *et al.* 2009, Moyle *et al.* 2012, Cai *et al.* 2019). Pellorneid species richness reflects their biogeographic history (Cai *et al.* 2020), being highest in the Sino-Himalayan Mountains (where the group originated) and in the Sundaland region. Though Wallacea lies immediately to Sundaland’s east, babblers colonised it more recently (crossing Wallace’s Line) and thus their species richness is lower in this region (Cai *et al.* 2020). Aspects of the natural history and ecology of babblers remain enigmatic (Ó Marcaigh *et al.* 2021b). As the Sulawesi Babbler is “smallish [and] relatively featureless” (Billerman *et al.* 2022) and tends to skulk in the understorey, it is of the kind of bird most likely to be overlooked (Diamond 1985, Gaston and Blackburn 1994). Its “mouse-like” behaviour (Billerman *et al.* 2022) suggests that, biogeographically, such understorey birds may have more in common with terrestrial mammals than more dispersive species.

If we are to understand how populations become species, it is natural that we must study populations as well as species. Variation below the species level provides the raw material for natural selection, as populations will begin to diverge before they evolve physiological barriers to reproduction

(Dobzhansky 1940). Therefore, targeting the species level and below allows us to study both current and past speciation (e.g. Brelsford and Irwin 2009, Everson *et al.* 2018). Different units of subspecific diversity have been used through scientific history, with examples including varieties (Linnaeus 1766), subspecies (Esper 1781, Mayr 1963), incipient species (Dobzhansky and Pavlovsky 1967), conservation units (Coates *et al.* 2018) or Evolutionarily Significant Units (ESUs) (Moritz 1994). Though division of subspecific diversity into units has often generated controversy, it is evident that this diversity is pivotal to evolution (O’Brien and Mayr 1991, Phillimore and Owens 2006). Indeed, while the species features in the title of evolutionary biology’s founding text (Darwin 1859), the subtitle refers to subspecific “races”. The Convention on Biological Diversity (1992) recognises biodiversity at the levels of genes, species, and ecosystems, but conservation at the gene level is hampered by lack of data, particularly in the biodiverse tropics (Bickford *et al.* 2007). Around 2% of vertebrate species are endemic to Wallacea (Myers *et al.* 2000), making it an urgent conservation priority that we understand Sulawesi’s endemism, both above and below the species level.

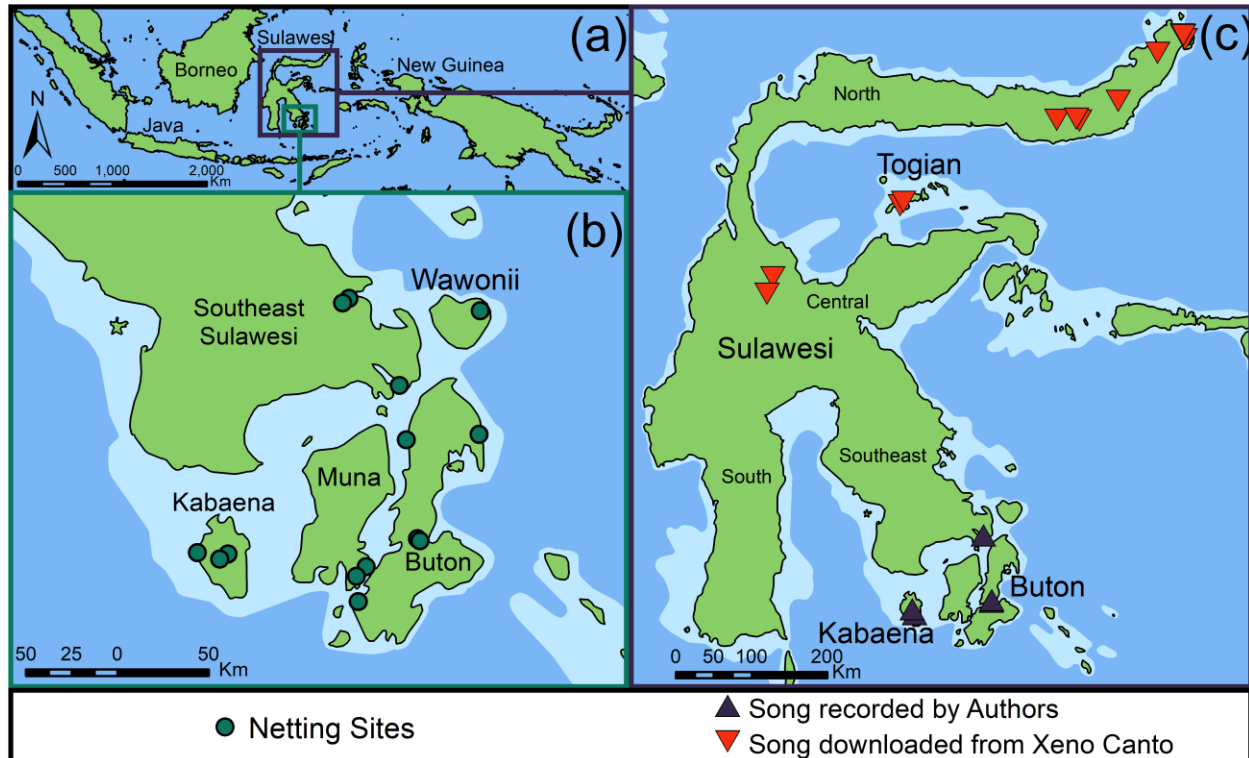
The Sulawesi Babbler provides one example of a bird species divided into subspecies based on plumage and other typological characteristics, where genetic data have been lacking. Current taxonomy (Gill *et al.* 2022) assigns all populations from Central and Southeast Sulawesi, as well as the land-bridge islands, to a single subspecies *P. c. rufofuscum* (Stresemann 1931). The wide range thus attributed to this subspecies crosses several present-day mountain ranges and seas. Central and Southeast Sulawesi were separate islands in the past, leading to differences between their monkeys, toads (Evans *et al.* 2003), hoofed mammals (Frantz *et al.* 2018), and trees (Trethowan *et al.* 2020). If flight allowed all birds to transcend this pattern, we would expect the widely distributed babblers designated as *rufofuscum* to present one genetically uniform population, following the current taxonomy. Alternatively, if the understorey lifestyle of babblers limited their gene flow in a manner similar to land mammals, we would expect to see diversification between Central and Southeast Sulawesi populations and potentially across land-bridge islands as well.

## 4.3 Methods

### 4.3.1 Sampling and data collection

We mist netted birds in Southeast Sulawesi (Figure 4.1, Table S4.1) and the land-bridge islands of Kabaena, Muna, Buton, and Wawonii, between 1999 and 2017. We used the methods outlined in Redfern and Clark

(2001) to photograph our birds (Figure S4.1) and measure a range of morphological traits including wing length (maximum chord), bill length (tip of bill to the base), and skull length (from back of skull to base of bill). Only adult birds measured by a single recorder (NMM) were used for morphological analyses. This gave a morphological sample size of 22 for Southeast Sulawesi, 18 for Kabaena, 6 for Muna, and 22 for Buton. Birds were also sampled for genetic analysis by taking a small number of contour feathers from the flank. Compared to other feather tracts, sampling of contour feathers minimises the risk of injury to the bird and avoids disrupting its flight ability and any plumage-based visual signals (McDonald and Griffith 2011). Genetic sample size was 5 for Southeast Sulawesi, 1 for Central Sulawesi, 8 for Kabaena, 6 for Muna, 5 for Buton, and 1 for Wawonii. We recorded the babbler songs using a Zoom H2 Handy Recorder, with a Sennheiser Me62 external microphone and a Telinga V2 parabolic reflector, and downloaded additional recordings from the website xeno-canto (<https://www.xeno-canto.org/>). The combined acoustic sample size from both sources was 15 for Southeast Sulawesi, 2 for Central Sulawesi, 12 for North Sulawesi, 2 for Togian, 17 for Kabaena, and 16 for Buton. Our acoustic sampling thus included three of the four Sulawesi Babbler subspecies recognised by Gill *et al.* (2022). These are the North Sulawesi subspecies *P. c. celebense* (Strickland 1849), the Togian subspecies *P. c. togianense* (Voous 1952), and *P. c. rufofuscum* which supposedly covers all of Central and Southeast Sulawesi and the land-bridge islands. Previous taxonomic treatments had a subspecies endemic to Southeast Sulawesi, named *sordidum* by Stresemann (1938) and renamed *improbatum* by Deignan (1964) as there was already a *sordidum* subspecies in the same genus. However, White and Bruce (1986) merged *improbatum* into *rufofuscum* based on their similar flank colour, and this move has been retained by Gill *et al.* (2022). The fourth subspecies is *P. c. finschi* (Walden 1876), endemic to South Sulawesi, but this taxon could not be included in this study as no sequences or recordings were available.



**Figure 4.1.**

- a) Map of central Indo-Pacific archipelago with study region outlined.  
 b) Map of Southeast Sulawesi with sampling sites marked with circles  
 c) Map of Sulawesi with song recording locations marked with triangles.

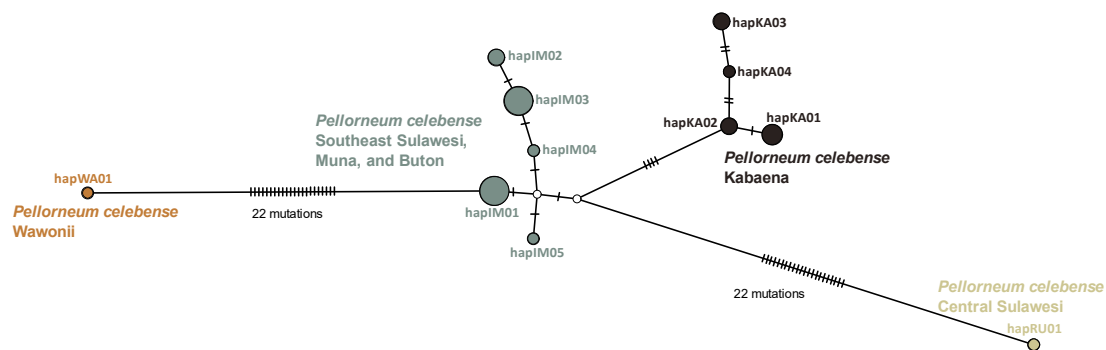
### 4.3.2 Genetic analyses

It has been demonstrated that divergence in mitochondrial genes correlates with speciation rate in tropical birds (Harvey *et al.* 2017). We used the mitochondrial DNA (mtDNA) genes NADH dehydrogenase subunits 2 and 3 (hereafter ND2 and ND3), to investigate population genetics of babblers. Barcoding approaches with mtDNA have proved successful in species delimitation (Hebert *et al.* 2004, Kerr *et al.* 2007, Hebert *et al.* 2016). While some evolutionary histories inferred from mtDNA differ from those inferred from nuclear DNA (Rubinoff and Holland 2005, Phillimore *et al.* 2008), and biogeographic patterns inferred from mtDNA can be obscured by introgression and male-mediated gene flow (Toews and Brelsford 2012), ND2 has shown a particularly high level of concordance with nuclear markers (Campillo *et al.* 2019), and studies on young radiations have found ND2 and ND3 to provide the best phylogenetic resolution (Andersen *et al.* 2015b). This makes them appropriate to study divergence at the level of populations and subspecies, as we aimed to do.

DNA was extracted from feathers using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, California, USA), following the manufacturer's instructions, but with the addition of 5 microlitres of 1M dithiothreitol (DTT). The DTT was added before the samples were vortexed prior to incubation, to break down the keratin from the base of the feather, which encased the genetic material. Polymerase Chain Reactions (PCRs) were carried out in 20 $\mu$ l reactions to target the ND2 and ND3 genes, using a touchdown cycling protocol to increase yield (Korbie and Mattick 2008). We amplified the ND3 gene using the L10755-F and DOC-ND3-R1 primer pair (Chesser 1999, O'Connell *et al.* 2019b), while ND2 was sequenced in two halves using established and novel internal and external primers (Table S4.2). The reactions were screened using 2% electrophoresis gels stained with GelRed (Biotium), then sequenced by GATC EuroFins using a Sanger sequencing protocol. These sequences were aligned using the ClustalW function in BioEdit (Hall 1999) and the ND2 and ND3 sequences were concatenated using Mesquite (Maddison and Maddison 2018), for a total of 1392bp. We used GenBank to obtain the sequences of the sole individual of *P. celebense* that had been sequenced previously (ND2 accession JN826691, ND3 accession JN826966), and those of its two sister species: *P. rostratum*, described by Blyth (1842) (ND2 JN826692, ND3 JN826967), and *P. bicolor*, described by Lesson (1839) (ND2 JN826690, ND3 JN826965). These sequences of the three *Pellorneum* species were published in Moyle *et al.* (2012), with the *P. celebense* material supplied by a bird from Banggai Province in Central Sulawesi. Our outgroup included other babbler genera (Cai *et al.* 2019) and outgroup taxa used by Moyle *et al.* (2012) (a full list with accession numbers is in Table S4.3). Our new ND2 and ND3 sequences have been deposited in GenBank under accession numbers MW387438- MW387487.

We used POPART (Leigh and Bryant 2015) to draw a TCS Network of the haplotypes we sequenced (Figure 4.2), to help visualise any potential population structure. The TCS algorithm uses an agglomerative approach, progressively combining clusters with one or more connecting edge (Templeton *et al.* 1992). A complete list of samples and their corresponding haplotypes is available in the Supplementary Information (Table S4.3). Only one representative of each ND2/ND3 haplotype was included in the Maximum Likelihood and Bayesian analyses. We used MEGA X (Kumar *et al.* 2018) to generate pairwise proportion differences (p-distances) between our concatenated ND2/ND3 haplotypes (Table S4.4) and to choose a nucleotide substitution model based on the Bayesian Information Criterion (BIC). It selected a Hasegawa-Kishino-Yano (HKY) model, which we used to perform Maximum Likelihood analysis with 1000 bootstraps and a level 5 Subtree-Pruning-Regrafting heuristic. We carried out Bayesian phylogenetic inference in MrBayes version 3.2.7 (Huelsenbeck and Ronquist 2001), using a HKY model with burn in set to 25%. This consisted of two

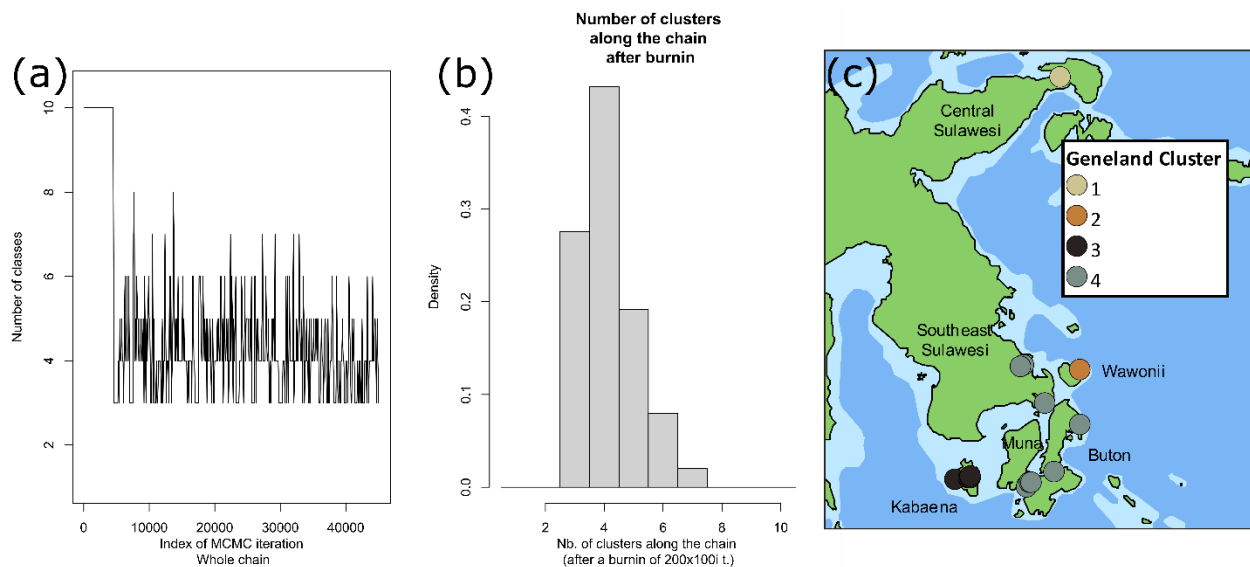
independent Markov chain Monte Carlo (MCMC) runs, with four chains per run, sampling every 1000 generations. We used TRACER version 1.7.1 (Rambaut *et al.* 2018) to assess convergence, accepting once average standard deviation in split frequencies (ASDSF) reached 0.01 and Effective Sample Size (ESS) of model parameters reached 200. These thresholds had been passed by 2 million generations. We exported a 50% majority rule consensus tree from MrBayes and merged it with the Maximum Likelihood tree using the *ggtree* R package (Yu *et al.* 2016). This merged tree is shown in Figure 4.3, with the outgroup collapsed and with Bayesian probabilities and ML bootstrap values displayed. A version with all outgroup taxa displayed is available in Figure S4.2.



**Figure 4.2.** Haplotype Network of concatenated babler ND2/ND3 sequences. Each coloured circle represents a haplotype, sized to represent the number of corresponding samples and coloured to represent our proposed delineation of populations. Each bar across the interconnecting lines represents one mutation. The small, unfilled white nodes represent hypothetical ancestral states.



basis in HWE, the package creators advise caution in the interpretation of clustering in non-recombining DNA, such as mtDNA (The Geneland Development Group 2020). Nevertheless, previous studies using the Geneland package have found clustering of the ND2 gene to agree with that of nuclear DNA (Trier *et al.* 2014, Klein *et al.* 2016). We ran a spatial MCMC algorithm on the 51 polymorphic sites of our concatenated babbler sequences for 45,000 iterations with thinning set to 100. We used Geneland’s “haploid” setting as our sequences were of mtDNA. This model’s output was then used to estimate the number of populations at HWE and assign each individual to a population (Figure 4.4). The Geneland model accomplishes this by dividing the study area using a Voronoi tessellation and using genetic and geographic distance to calculate the probability of individuals originating from the same population (Guillot *et al.* 2005a). As all sequenced birds came from Central and Southeast Sulawesi and are currently grouped as the *P. c. rufofuscum* subspecies, the current taxonomy of the species would be supported if this analysis found only one cluster. By the same token, multiple clusters would indicate more genetic populations than captured by current taxonomy.



**Figure 4.4.**

**a)** Plot of the Geneland MCMC estimating the number of clusters, i.e. populations at HWE in the babblers of Central and Southeast Sulawesi.

**b)** Histogram summarising the MCMC results and finding four clusters to be the most likely result.

**c)** Map of babbler concatenated sequences with the clusters they were assigned to by the Geneland analysis.



#### 4.3.4 Acoustic analysis

Comparative analysis of bird song has come to play a central role in species delimitation and integrative taxonomy, as differences in song have been shown to lead to reproductive isolation and speciation (Isler *et al.* 1998, O'Reilly *et al.* 2018). The Sulawesi Babbler often sings in duet, with the “main song” produced by one individual (presumed to be the male of a pair) answered with a distinct vocalisation from the presumed female (Billerman *et al.* 2022). We analysed the main, “male” song, and not the answering song of the presumed female, as there were more uninterrupted song bursts available and male song is more likely to be relevant to speciation, as mate choice by females based on male song may reinforce reproductive isolation if song diverges between populations (Catchpole 1987). We used Raven Pro version 1.6 (Center for Conservation Bioacoustics 2019) to create spectrograms from the babbler recordings and measured these using on-screen cursors to collect data. Our acoustic dataset consisted of standard spectral and temporal song traits: peak frequency, duration, minimum frequency, maximum frequency, bandwidth, number of notes, and pace (Tobias *et al.* 2010, O'Connell *et al.* 2019c). To aid in visualisation, contrast and brightness were set to an equal value and the “Jet” colormap was selected; all other settings were left at their defaults (Ng *et al.* 2016). To account for intra-individual variation, intra-individual means were calculated from a minimum of two independent bursts of song (average of 8.1 songs, range 2-26) (Ng *et al.* 2016). These means served as our sample points (Supplementary File 2).

To give an impression of whether babbler populations could be distinguished by song, we used the “random forest” algorithm in WEKA version 3.8.4 (Frank *et al.* 2016). This allowed us to look for clusters independently in our different datasets. As Geneland and our haplotype network and phylogenetic tree had suggested certain patterns of genetic clustering in the purported *rufofuscum* subspecies, we were able to test whether this was reflected by song differences while also extending the comparison to two other recognised babbler subspecies (*celebense* from North Sulawesi and *togianense* from Togian).

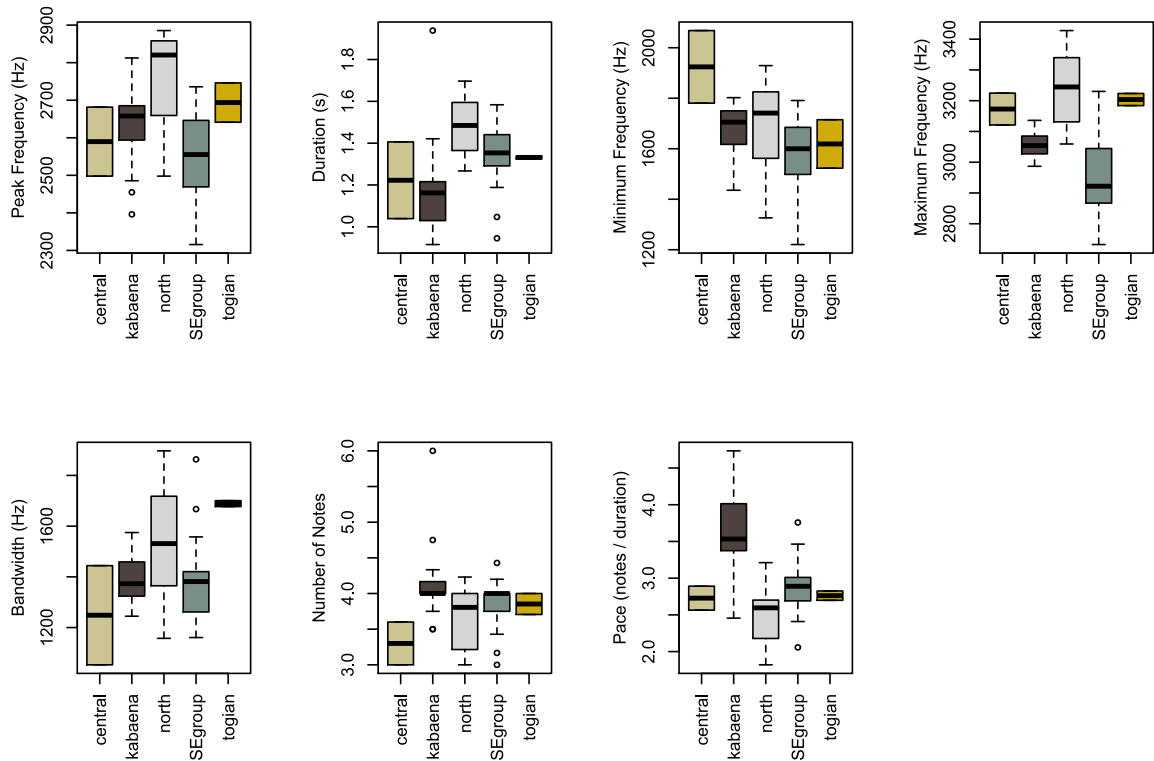
A random forest is a supervised classification algorithm which uses a series of decision trees to partition the dataset. A random subset of input variables are used to create bootstrapped subsets of training data to combine into a final model, splitting the data in a way that is unbiased and robust (Breiman 2001). Random forests have been used to diagnose samples by their origin in other fields, such as geology (Dornan *et al.* 2020) and botany (Finch *et al.* 2017), and are increasingly used in similar fashion in species delimitation and population genetics (Derkarabetian *et al.* 2019, Smith and Carstens 2020). Diagnosability is key to defining taxa, and is emphasised in particular by the Phylogenetic Species Concept (Archer *et al.* 2017). We used the “training set” setting for WEKA's random forest. A subset of the babbler data was used to train the

algorithm, by dividing these training examples by subspecies and additional divisions suggested by the genetic and phylogeographic analyses. In keeping with the current taxonomy, we labelled birds from North Sulawesi as *celebense*, those from Togian *togianense*, and those from Central Sulawesi *rufofuscum*. Based on genetic divisions we grouped together the birds from Southeast Sulawesi and Buton as the “southeast group” and gave separate labels to those from Kabaena. We did not have songs from Wawonii or Muna (Table 4.1). The algorithm then attempted to classify the rest of the data based on differences in the training set. For comparison, we also ran Random Forest analyses which tried to group the birds by island, i.e. with Buton, Southeast Sulawesi, and Muna treated separately.

**Table 4.1:** Number of individual babblers from each area available for each analysis. Mainland Sulawesi areas are bolded, offshore islands are italicised.

<b>n</b>	<b>Southeast Sulawesi</b>	<b>Central Sulawesi</b>	<b>North Sulawesi</b>	<i>Togian</i>	<i>Kabaena</i>	<i>Muna</i>	<i>Buton</i>	<i>Wawonii</i>
<b>DNA</b>	5	1	0	0	8	6	5	1
<b>Morphology</b>	22	0	0	0	18	6	22	0
<b>Song</b>	15	2	12	2	17	0	16	0

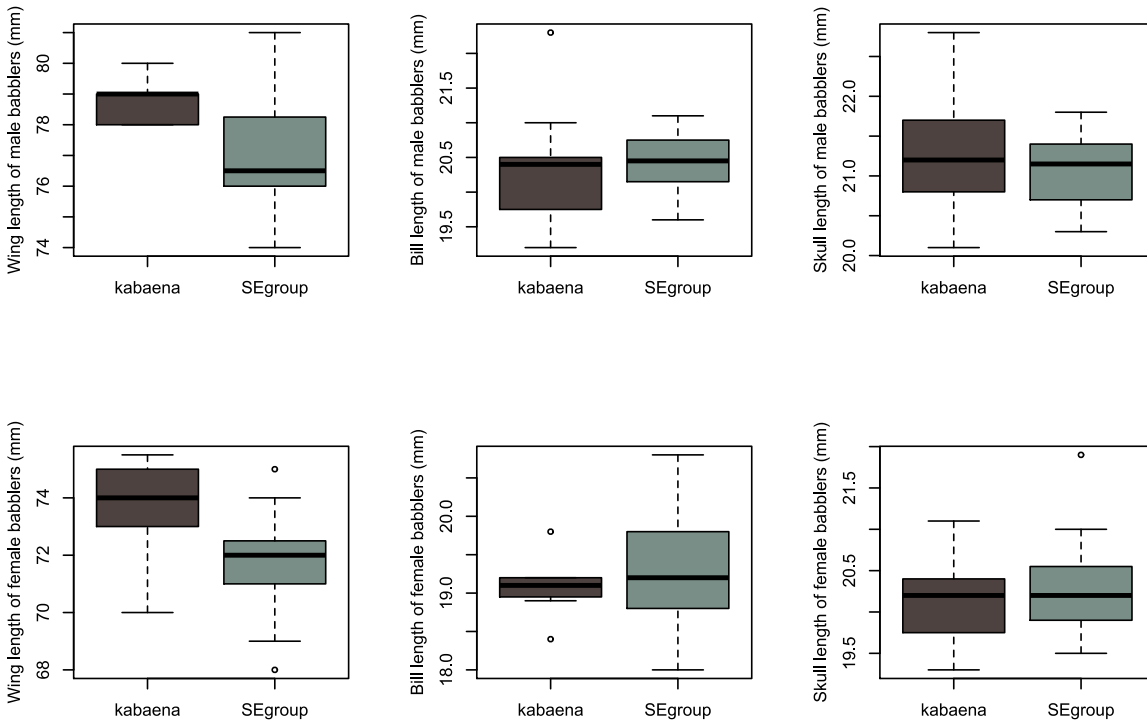
To confirm the pattern suggested by the Random Forest analysis, we used R to carry out a multivariate ANOVA (or MANOVA) on the acoustic data to see if differences between songs of populations were statistically significant. This MANOVA tested the difference between the North Sulawesi, Togian, Central Sulawesi, “southeast group” (Southeast Sulawesi and Buton) and Kabaena populations in peak frequency, duration, minimum frequency, high frequency, bandwidth, number of notes, and pace. We visualised the acoustic data using box plots (Figure 4.5).



**Figure 4.5.** Box plots of acoustic traits of Sulawesi Babblers from Central Sulawesi, Kabaena, North Sulawesi, the Southeast Group (including mainland Southeast Sulawesi and Buton), and Togian.

### 4.3.5 Morphological analyses

Only adult birds measured by NMM were included in morphometric analyses, and male and female babblers were analysed separately as males are considerably larger (Ó Marcaigh *et al.* 2021a). The morphometric analyses thus included only birds from the Kabaena ( $n = 18$ ) and “southeast group” ( $n = 50$ ) populations, where the southeast group comprised Southeast Sulawesi, Muna, and Buton. We selected wing length, bill length, and skull length for the morphological analyses as they are independent variables that correspond to different aspects of the birds’ ecology (Ó Marcaigh *et al.* 2021a). As with the acoustic data, we first carried out a random forest classification to see if these populations could be distinguished based on these three traits, then a MANOVA to test whether the differences were statistically significant. We constructed box plots to visualise the morphological data (Figure 4.6).



**Figure 4.6.** Box plots of morphological traits of Sulawesi Babbler from Kabaena and the Southeast Group (including mainland Southeast Sulawesi, Buton, and Muna). The top row of box plots represent the male babbler, the bottom row the females.

### 4.3.6 Tobias scoring

We subjected our putative babbler populations to the quantitative scoring criteria outlined by Tobias *et al.* (2010), where populations with a “score” of 7 or more are seen as deserving of species status. This score is a combination of differences in morphology, vocalisations, plumage, ecology or behaviour, and geography. The geography category awards points for situations of sympatry and hybrid zones, and so does not apply to island populations. We had no data on ecology or behaviour, and our photographs of live birds in the field (Figure S4.1) could not demonstrate plumage differences due to the variation within the populations and varying light conditions, so we were unable to score those areas. Therefore we calculated a partial Tobias score for the Kabaena population on acoustics and morphology, using the “effsize” package in R (Torchiano 2020) to calculate Cohen’s *d*. Effect sizes are more suitable than p-values for informing taxonomic judgements as they are less correlated with sample size (Tobias *et al.* 2010). We used male morphology for

our Tobias score because the acoustic score was based on the male song. We also made similar comparisons with female morphology and the effect sizes were such that the score would have been the same.

## 4.4 Results

### 4.4.1 Genetics and phylogeography

Genetic analysis provided evidence of population structure within the ostensible *P. c. rufofuscum* subspecies of Central and Southeast Sulawesi. Southeast Sulawesi shared concatenated ND2-ND3 haplotypes with Muna and Buton, but not with Wawonii, Kabaena, or Central Sulawesi (Figure 4.2). For that reason, in further analyses we grouped Southeast Sulawesi, Buton, and Muna as the “southeast group”, separate to Central Sulawesi. The Central Sulawesi haplotype (HapRU01) was between 1.7% and 1.9% different from the southeast group haplotypes, while it was 2.4% different from the Wawonii haplotype (hapWA01) and 1.9-2.2% different from the Kabaena haplotypes (Table S4.4). Babblers from Kabaena and the southeast group are distinct, monophyletic clusters on the phylogenetic tree (Figure 4.3), each diverging from a common ancestor.

The Geneland MCMC analysis found best support for a 4-cluster model (Figure 4.4), followed by a 3-cluster model. This indicates that there is more population structure in these babbler populations than is recognised by the current taxonomy, where all of these birds are considered to belong to one subspecies (*P. c. rufofuscum*). Furthermore, its proposed populations line up with the divisions suggested by the phylogenetic tree, with the Kabaena, Wawonii, southeast group, and Central Sulawesi clusters all apparent (Figure 4.4c).

The ABGD analysis found the most support for three species in *Pellorneum* (*P. rostratum*, *P. bicolor*, and *P. celebense*), with five different barcode gaps between 0.8% and 6% difference in concatenated ND2-ND3 producing this grouping. At the lowest prior intraspecific divergences, however, the analysis calculated the barcode gap distance to be 0.1% and with this it created six groups: *P. rostratum*, *P. bicolor*, *P. celebense* from Central Sulawesi, *P. celebense* from Wawonii, *P. celebense* from Kabaena, and *P. celebense* from the southeast group.

#### 4.4.2 Acoustic results

The Random Forest analysis on the seven acoustic traits had a 100% success rate in classifying birds according to our population divisions of Togian, North Sulawesi, Central Sulawesi, the “southeast group” (Southeast Sulawesi and Buton), and Kabaena. The Kappa statistic, F-Measure and ROC Area for each division was equal to 1, indicating an optimal classifying model. MANOVA on the seven traits agreed, finding a statistically significant difference between the same population divisions ( $p < 0.001$ ). The full output from this MANOVA is in the Supplementary Information (Table S4.5). The Random Forest acoustic analysis which treated each island individually was much less successful, as it classified 31 birds incorrectly (48%). The Kappa statistic was 0.37, indicating a suboptimal model.

#### 4.4.3 Morphological results

Once juvenile birds were excluded from the morphological dataset, this left only the southeast group and the Kabaena population. Even so, the Random Forest analysis categorised each dataset with 100% accuracy and with Kappa statistics, F-Measures and ROC Areas equal to 1. The MANOVA on male babblers from the southeast group and from Kabaena found that they were statistically significantly different in wing length, bill length and skull length ( $p < 0.05$ ), as did the MANOVA on female babblers from the same two populations, using the same traits ( $p < 0.05$ ). The full outputs from these MANOVAs are in the Supplementary Information (male babblers in Table S4.6, females in Table S4.7). When treating each island separately, the accuracy of morphological Random Forest analysis on males declined to 35%, while accuracy on females was reduced to 40%.

#### 4.4.4 Tobias scoring

We applied the Tobias species delimitation system (Tobias *et al.* 2010) to our division between the Kabaena population and the southeast group (consisting of Southeast Sulawesi, Muna, and Buton), because this division had been supported by all of our previous analyses. This system allows use of one spectral acoustic character and one temporal in distinguishing between bird populations. Peak frequency was the most divergent spectral character between Kabaena and the southeast group, with a Cohen’s  $d$  of 0.36. Pace was the most divergent temporal character, with a Cohen’s  $d$  of 1.77. These constitute “minor” differences under the Tobias system, scoring 1 point each.

Two morphological traits may be included in calculating a Tobias score: that showing the largest increase, and that showing the largest decrease. When comparing birds from Kabaena to those from the southeast group, these were wing length (Cohen's  $d$  of 1.28) and bill length (Cohen's  $d$  of -0.25). The system ranks these effect sizes as "minor" and awards 1 point for each of them. Added together, these give a Tobias score of 4 for the Kabaena population when compared to babblers from Southeast Sulawesi, Buton, and Muna.

## 4.5 Discussion

Wallace (1887) set the priorities of biogeographers for centuries when he wrote that "The continental islands, still attached as they are to the base of the mainland, are to all intents and purposes a portion of the continent, as well in structure as in the forms of animal and vegetable life which they afford. It is in the oceanic islands that we should meet with limited and peculiar types." Oceanic islands continue to receive the most attention in speciation studies today (Tobias *et al.* 2020). While continental land-bridge islands indeed harbour fewer endemic species, their populations may yet represent unique components of the species, important for both current biodiversity and future evolution. The fact that land-bridge islands are numerous and tend to be richer in species makes their populations more interesting still (Meiri 2017, Tobias *et al.* 2020). It is apparent that babblers on the land-bridge islands of Kabaena and Wawonii have diverged from mainland populations in the brief time since these landmasses became physically disconnected. Previous work has shown that babblers on the land-bridge islands of Sulawesi exhibit stronger sexual dimorphism than those on the mainland (Ó Marcaigh *et al.* 2021a). This chapter adds that babblers on Kabaena are distinct in acoustics, morphology, and mtDNA, and that the Wawonii population is strongly divergent in mtDNA. Combined with the division we found between Southeast Sulawesi and Central Sulawesi, this indicates that the subspecies *Pellorneum celebense rufofuscum* is actually comprised of four divergent lineages.

The babblers of Southeast Sulawesi were formerly recognised as a distinct subspecies, *P. c. improbatum*, originally described by Stresemann (1938) from a type specimen from Lalolai in Southeast Sulawesi (latitude -4.05, longitude 121.88). Though this taxon was not recognised by White and Bruce (1986) and Gill *et al.* (2022), our analyses have reaffirmed that the babblers of the southeast group (Southeast Sulawesi, Buton, and Muna), do in fact represent an evolutionarily distinct population. The Southeast population has diverged from Central Sulawesi both acoustically and morphologically, mirroring the patterns of endemism seen in trees, monkeys, toads, and hoofed mammals (Evans *et al.* 2003, Frantz *et al.* 2018,

Trethowan *et al.* 2020). This supports the hypothesis that these understory birds are as disinclined to disperse as are non-volant organisms, with corresponding impacts on evolutionary trajectories. This is in keeping with findings from other understory babbler species, which have also diverged genetically in areas that were recently connected by land bridges (Cros *et al.* 2020).

The distances involved would appear to be too short to fully explain the divergence seen. Kabaena is the most distant of the land-bridge islands at around 18km from the Sulawesi mainland (Robinson-Dean *et al.* 2002), still a relatively short distance in terms of speciation. Wawonii is only 7km from Sulawesi, comparable to Buton which is 6km from the mainland at its closest point. Muna is separated from Buton by only 0.6km. Habitats on Kabaena and Wawonii may be isolated by geology more than distance, as both islands are dominated by a distinct ultramafic geology that produces soils poor in nutrients and rich in phytotoxic minerals (Galey *et al.* 2017). Such soils present distinct selection pressures for organisms and are noted for very high levels of plant endemism (Anacker 2014), which would in turn present a distinct evolutionary environment for animals including babblers. Genetic erosion could also play a role: Kabaena and Wawonii are smaller than Muna and Buton, creating the potential for genetic bottlenecks. Populations of other taxa on Kabaena and Wawonii have been noted for their distinctness from neighbouring islands, showing the potential for evolutionary and ecological divergence despite the short time scale. Tweedley *et al.* (2013) observed a pronounced difference between the composition of the freshwater fish faunas of Kabaena and Buton. Trethowan *et al.* (2020) found the tree communities of Wawonii to comprise different species than those of Central Sulawesi.

Within birds, the patterns of evolutionary divergence in the region can be linked to life history. *Zosterops* white-eyes (Vigors and Horsfield 1826) are famous for their dispersal abilities, though more isolated populations are known to develop behavioural flightlessness (Moyle *et al.* 2009). *Zosterops* populations on Kabaena and Wawonii show no sign of divergence (O'Connell *et al.* 2019c). The Olive-backed Sunbird *Cinnyris jugularis* (Linnaeus 1766, p.185) and grey-sided flowerpecker *Dicaeum celebicum* (Müller 1843) both inhabit marginal habitats such as forest edges, scrubland and mangroves (Billerman *et al.* 2022), a trait that makes a bird more likely to cross habitat gaps and open spaces (Burney and Brumfield 2009). They too share populations across Kabaena and Sulawesi (Kelly 2014, Kelly *et al.* 2014, O'Connell *et al.* 2019c). The Red-backed Thrush *Geokichla erythronota* (Sclater 1859) presents a notable contrast to these species, having diverged strongly enough on Kabaena to produce an endemic subspecies *G. e. kabaena* (Robinson-Dean *et al.*



2002). Like the Sulawesi Babbler, the Red-backed Thrush is a bird of the forest understorey, and the two species have even been observed foraging together (Billerman *et al.* 2022).

Evolutionary divergence within the babblers of Sulawesi and its land-bridge islands can be compared to that between more geographically isolated lineages. This study found Moyle *et al.*'s (2012) *Pellorneum rostratum* sequence from Borneo to be 7% different from all *Pellorneum celebense* haplotypes. Borneo and Sulawesi have been separated for the duration of their existence by the Makassar Strait, part of the permanent barrier of deep water known to biogeographers as Wallace's Line (Wallace 1880, Tweedley *et al.* 2013). This 7% difference in concatenated ND2-ND3 is thus the result of millions of years of evolution, while the populations of Sulawesi and its land-bridge islands have developed a p-distance around 1/3 of this in only 12,000 years or so (Table S4.4). This illustrates the remarkable speed with which speciation can act on dispersal-limited species, as well as the impact of genetic drift on these relatively small island populations.

Our work highlights the importance of units below the species level to the evolutionary potential of the Sulawesi Babbler. This joins a long-standing debate in evolutionary biology, where approaches to species and subspecies often generate controversy. Earlier naturalists named many subspecies based on typological traits and came in for some contemporary criticism (Wilson and Brown 1953). While modern methods overturned many of their designations, they reaffirmed others: Hartert (1903), for example, named two endemic species from the Wakatobi islands which were later demoted to subspecies (White and Bruce 1986), before being confirmed as reproductively isolated species a century later (Kelly *et al.* 2014, O'Connell *et al.* 2019c). The higher levels of the Linnaean hierarchy were prioritised early in the molecular age, but a broad consensus persisted that the subspecies concept is useful in naming distinct populations with geographical boundaries between them, using multiple lines of evidence (Wiens *et al.* 1982). Phillimore and Owens (2006) found that island subspecies are the most likely to reflect evolution accurately and suggested that subspecies can aid conservation in the tropics. We have sought to follow these recommendations by analysing geographically delimited populations on tropical islands using multiple lines of evidence.

Modern ornithologists delimit species with an integrative approach based on comparison to recognised species and ultimately derived from the Biological Species Concept (Tobias *et al.* 2010). This integrative approach is particularly important when genetic data are absent or, as here, based on relatively low genetic sample sizes. Based on two of the five scoring criteria, the Kabaena population attains a Tobias

score of 4, three points short of species status. Despite the lack of data on plumage, ecology, and behaviour, this partial score was more than half of that required to identify a distinct species, strongly suggesting that the Kabaena birds and the southeast group represent separate populations. The Wawonii population cannot be scored as it lacks acoustic and morphological data. Note that this system does not incorporate genetic data. We thus propose the distinct babbler populations as subspecies and not as independent species. Our ABGD analysis supports this conclusion, as it separated these four *P. celebense* populations from one another using a small barcode gap of 0.1% difference in concatenated ND2-ND3, appropriate for splits between subspecies. When the barcode gap was between 0.8% and 6%, similar to that between ND2 sequences of other bird species (e.g. Pellegrino *et al.* 2017, O'Connell *et al.* 2019c), ABGD grouped *P. celebense* together as a single species. Fuller Tobias assessments, incorporating plumage, ecology, and behaviour, might lead to other conclusions.

We thus recommend that the subspecies *Pellorneum celebense improbatum* be recognised for babblers from Southeast Sulawesi, Buton, and Muna, as these are genetically and acoustically divergent from the *P. c. rufofuscum* population of Central Sulawesi. We propose that babblers from Kabaena be named as a new subspecies for their genetic, acoustic, and morphological divergence from Southeast Sulawesi, Buton, and Muna. This would require collection of a voucher specimen from Kabaena, which could then be analysed genetically to ensure that it belongs to the divergent population detailed here. Historical collectors did not visit Kabaena (White and Bruce 1986) and as a result very few species have ever been collected there, in fact the island was almost unknown ornithologically until recently (O'Connell *et al.* 2017). Lack of museum material should not delay conservation (O'Connell *et al.* 2020a). We therefore suggest the provisional name of *P. c. kabaena* for the Kabaena babblers and recommend that they be subjected to formal description. The Wawonii population shows strong divergence in mtDNA, but as this is based on one sample and lacks acoustic or morphological data, we recommend that it be studied in more detail before a taxonomic judgement is made.

Separate to any taxonomic revision, characterising the divergence of populations on Wallacean islands is key to our understanding of how speciation creates the biodiversity of this global hotspot. The concept of the Evolutionarily Significant Unit, or ESU, aims to sidestep taxonomic debate by targeting conservation at lineages that are evolving independently, regardless of how these are assigned to taxa (Ryder 1986, Moritz 1994, Coates *et al.* 2018, Neal *et al.* 2018). We believe that four independent ESUs are present

in the populations currently assigned to this one babbler subspecies, one each from Central Sulawesi, Southeast Sulawesi, Kabaena, and Wawonii.

These populations of the supposed *rufofuscum* subspecies have undergone divergent evolution, despite being physically capable of maintaining gene flow between the land-bridge islands and the mainland. As Mayr (1969) noted, “Most tropical birds are highly sedentary and respect water barriers to a high degree”. This also applies to the division between Central Sulawesi and Southeast Sulawesi, which were separated by water barriers for much of their geological history. Taxonomists should be wary of lumping the taxa of these two “areas of endemism” (Evans *et al.* 2003) together. Just as our understanding of a species and its evolution is improved by analysing its constituent populations, there is an urgent need to consider which areas within the threatened biodiversity hotspots are most important in generating this biodiversity through speciation. Sometimes these areas are imperilled by the very traits that make them evolutionarily significant: the ultramafic nickel deposits of Kabaena and Wawonii also make them attractive to large-scale mining (Morse 2019b, a). Time is therefore running out to build a full picture of the biodiversity of these islands and their evolutionary dynamics.

## Chapter 5 - Tramps in transition: genetic differentiation between populations of an iconic "supertramp" taxon in the Central Indo-Pacific

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*"... Ultimate freedom. An extremist. An aesthetic voyager whose home is the road. ... Ten days and nights of freight trains and hitchhiking bring him to the great white north. No longer to be poisoned by civilization he flees, and walks alone upon the land to become lost in the wild.*

ALEXANDER SUPERTRAMP

MAY 1992"

- Part of a message written inside abandoned Fairbanks Bus 142 by Christopher McCandless, via Jon Krakauer (1996)

**Author Contribution:** I conceived this study with Darren O’Connell, David J. Kelly, and Nicola Marples. I led the writing, with input from David, Nicola, and Darren. David and Nicola led the field work, with contributions from Darren, Adi Karya, Kangkuso Analuddin, and me. I carried out the labwork, with help from Naomi Lawless and Niamh Doyle. Caroline McKeon measured the geographic distances. I analysed the data and produced the figures.

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**Chapter Illustration:** Island Monarch, from the original species description by Temminck (1827).

## 5.1 Summary

The Island Monarch (*Monarcha cinerascens*) was an original example of the “supertramp strategy”.

This involves well-developed dispersal specialisation, enabling a species to colonise remote islands but leaving it competitively inferior. Supertramps are hypothesised to be excluded from larger islands by superior competitors. It is the only original Melanesian supertramp to occur in Wallacea, home also to the sedentary Pale-blue Monarch (*Hypothymis puella*). We interrogate the supertramp strategy and its biogeographical underpinnings by assessing the population structure of these two monarchs. We sampled island and Pale-blue Monarchs in Wallacea, collecting DNA and morphological data. We investigated monarch population structure by applying ABGD and Bayesian and Maximum Likelihood methods to their ND2 and ND3 genes. We constructed linear models to investigate the relationships between genetic divergence, dispersal ability, and island area, elevation, and isolation. Wallacea’s deep waters restrict gene flow even in a supertramp, as the Wallacean and Melanesian Island Monarchs are likely separate species (mean genetic distance: 2.7%). This mirrors the split of the Pale-blue Monarch from Asia’s Black-naped Monarch (*Hypothymis azurea*). We found further population structure within Wallacean and Melanesian Island Monarch populations. Their genetic divergence was related to elevation, area, and isolation of islands, as well as dispersal ability of birds. However, dispersal ability was independent of island elevation and area. Rather than being *r*-selected on small, disturbance-prone islands, our results support the view that the Island Monarch’s supertramp lifestyle is a temporary stage of the taxon cycle, i.e. supertramps may transition into resident species after colonisation. Our models suggest that more dispersive monarchs reach more distant islands, and divergence is promoted on islands that are more distant or larger or more permanent, without selection against dispersal ability *per se*. We suggest that supertramp lifestyle helps determine the distribution of species across islands, not necessarily the divergence occurring thereafter.

## 5.2 Introduction

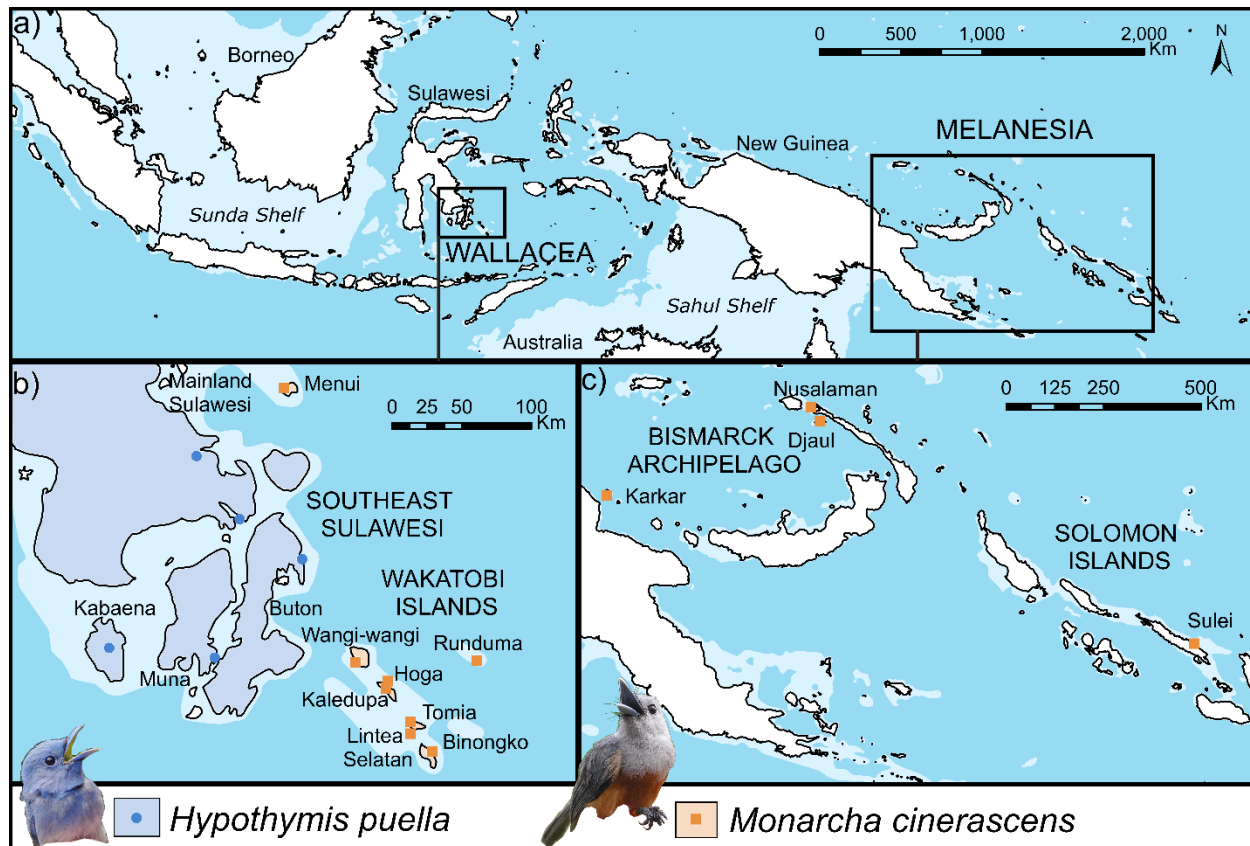
Animals vary in their ability to move or disperse through the environment, and these differences affect not only the range size of individual species (Sheard *et al.* 2020), but broad patterns of speciation (Smith *et al.* 2014, Manthey *et al.* 2020) and even the biogeographic division of life between regions of the Earth (White *et al.* 2021). The word “supertramp” originated in the autobiography of migrant worker WH Davies, “a free knight of the highway [who] lived like a pet bird on titbits” (Shaw 1908) and was later introduced into the scientific literature by Diamond (1974) to describe the life history strategies of nine bird species with wide distribution ranges, which occur only on small, isolated, or recently defaunated islands. Diamond attributed

the life history strategy of these nine supertramps, which he studied on the Bismarck and Solomon islands in Melanesia (Mayr and Diamond 2001), to an extreme form of  $r$  selection (MacArthur and Wilson 1967), which selects “for dispersal ability and reproductive potential at the expense of competitive ability” (Diamond 1974). Small islands are prone to disturbance and so these conditions would select for highly dispersive birds, but due to their low competitive ability these species would be excluded from larger islands inhabited by stronger competitors (Diamond 1974).

Diamond’s nine supertramps comprised a varied assortment of species: in updated taxonomy (here I follow Gill *et al.* (2022)) they include three doves (*Ptilinopus solomonensis*, *Ducula pistrinaria*, and *Macropygia mackinlayi*), a kingfisher (*Todiramphus tristrami stresemanni*), and five passerines: one monarch flycatcher (*Monarcha cinerascens*), one white-eye (*Zosterops griseotinctus*), one whistler (*Pachycephala melanura dahli*), and two honeyeaters (*Myzomela sclateri* and *Myzomela pammelaena*). This disparate grouping allows us to compare supertramps to relatives with contrasting life histories. The “supertramp” label has since been applied to additional Melanesian birds by Mayr and Diamond (2001), and to other birds and non-avian taxa by numerous authors (e.g. Cronk *et al.* 2005, Balke *et al.* 2009, Cibois *et al.* 2011, Pedersen *et al.* 2018, Pepke *et al.* 2019). The supertramp strategy has been examined using genetic studies on white-eyes (Linck *et al.* 2016) and cuckoo-shrikes (Pedersen *et al.* 2018, Pepke *et al.* 2019). Linck *et al.* (2016) found population structure in the Louisiade white-eye, one of Diamond’s original supertramps, with populations on larger and higher islands the most genetically distinct. In their studies on cuckoo-shrikes, Pedersen *et al.* (2018) and Pepke *et al.* (2019) found some young taxa to be widespread and undifferentiated. They dubbed these supertramps and contrasted them with older taxa which show greater genetic differentiation between populations. Linck *et al.* (2016) and Pepke *et al.* (2019) built a case that, rather than being selected for by  $r$  selection on small islands, the supertramp strategy is temporary and will be replaced by a sedentary lifestyle over time. We sought to build on this view by incorporating data on dispersal ability and extending it to another of the original supertramps, the Island Monarch (*Monarcha cinerascens*) (Temminck 1827), using data from Wallacea as well as some of the Melanesian islands where the theory originated.

Most of Diamond’s original supertramps are confined to Melanesia. The Island Monarch is unique among them in that its range stretches to Wallacea in central Indonesia, a region of deep water between the Sunda and Sahul shelves (Ali and Heaney 2021), full of small and isolated islands (Figure 5.1). As in Melanesia, the deep waters of Wallacea act as a barrier to the dispersal of many species, offering us a “natural

laboratory” (Whittaker *et al.* 2017) for the study of the movements of this supertramp. Though present in both Wallacea and Melanesia, it is absent from New Guinea and other large islands in between, a peculiarly disjunct distribution shared with 23 other dispersive bird species (White *et al.* 2021). Despite their dispersal abilities, the Island Monarch and these other 23 species do not occur west of Sulawesi and are thus subject to classical biogeography’s most famous boundary line (Wallace 1863, Ali and Heaney 2021). “Wallace’s Line” marks the edge of the Sunda Shelf, suggesting that this boundary between deep and shallow water is significant to even the most dispersive species.



**Figure 5.1.**

a) Map of Indo-Pacific with the Wallacea and Melanesia regions labelled. Seas deeper than 200 metres are drawn in a darker blue.

b) Map of Southeast Sulawesi and associated islands in Wallacea. Pale-blue Monarch range is shown in blue, Island Monarch range in orange, both based on the authors’ observations in the region. Islands where we sampled monarchs are labelled. Pale-blue Monarch sampling sites are marked as blue circles, Island Monarch sampling sites as orange squares. There were no islands on which both species occurred.

c) Map of Melanesia. Islands where Andersen *et al.* (2015a) sampled Island Monarchs in the Bismarck and Solomon archipelagos are labelled and marked with orange squares.

Pale-blue Monarch photo taken by Emma Shalvey, Island Monarch by DJK.

To maintain gene flow across a distribution that spans Wallacea and Melanesia, the Island Monarch must have the greatest dispersal abilities of all the supertramps considered by Diamond (1974). Alternatively, this apparently wide distribution could be a relic of outdated taxonomy: since Diamond's work, some of the Melanesian supertramps have been split from Wallacean relatives, like the Melanesian kingfisher (*Todiramphus tristrami*, formerly *Halcyon chloris stresemanni*). Indeed, recent investigations suggest that the Island Monarch may warrant division into multiple species (Eaton *et al.* 2021). If the Melanesian and Wallacean Island Monarchs are separate species, despite their similar appearances (i.e. cryptic species), we would expect them to exhibit genetic divergence equivalent to that between other *Monarcha* species. The identification of such cryptic species is vital to developing a full understanding of biodiversity, in this region and around the world (Fišer *et al.* 2018). As Wallacea has been relatively little studied, distributions of species in this region have also been unclear. Older distributions for the Island Monarch (e.g. del Hoyo *et al.* 2006) marked it as a year-round resident on larger Wallacean islands like Sulawesi and Timor and absent from the smaller Menui (also spelled Manui) and the Wakatobi Islands (also called Tukangbesi). Mayr and Diamond (2001) allow for cases where a species exhibits a supertramp distribution in one archipelago, while occurring on both small and large islands in another (this they term a tramp distribution). However, recent work has clarified that the Island Monarch exhibits a true supertramp distribution in Wallacea, as it does in Melanesia, being resident only on small islands, including Menui and the Wakatobi, and not on larger islands (Martin *et al.* 2012, Rheindt *et al.* 2014, O'Connell *et al.* 2017, Monkhouse *et al.* 2018, O'Connell *et al.* 2019d, O'Connell *et al.* 2020b, Eaton *et al.* 2021).

Wallacea is famed as a transition zone between Asian and Australo-Papuan faunas (Ali and Heaney 2021). The Island Monarch, representative of the Australo-Papuan genus *Monarcha*, is joined in Wallacea by the Asian monarch genus *Hypothymis* in the form of the Pale-blue Monarch (*Hypothymis puella*). This species was described by Wallace (1862), then lumped with the Black-naped Monarch (*Hypothymis azurea*) by Rand (1970) and split once again by Fabre *et al.* (2012). This split has been upheld by Gill *et al.* (2022). The Black-naped Monarch occurs all over the Sunda Shelf and as far west as India, so this split shows Wallacea's water barriers can block the dispersal of even widespread species. Like many Indo-Pacific bird families, the systematics of monarchs remained unclear until molecular sampling improved in recent years (Andersen *et al.* 2015a). Andersen *et al.* (2015a) placed *Hypothymis* and *Monarcha* in two separate clades of the Monarchidae, providing DNA sequences for four Island Monarchs from Melanesia. The Pale-blue Monarch is endemic to a few of Wallacea's larger islands, including Sulawesi and nearby land-bridge islands Kabaena,



Muna, Buton, and Wawonii (Figure 5.1). These islands were all connected to one another, and to the Sulawesi mainland, during geologically recent glaciations (Nugraha and Hall 2018). As the only other monarch in this part of Wallacea, the Pale-blue Monarch could fill the role of the *K*-selected sedentary competitor excluding the supertramp Island Monarch from larger islands. Such a role was outlined for other non-supertramp monarch species in Melanesia by Mayr and Diamond (2001) and a similar exclusionary relationship was hypothesised between the whole *Monarcha* and *Hypothymis* genera by Rand (1970).

Unlike the land-bridge satellite islands occupied by the Pale-blue Monarch, the nearby Wakatobi and Menui islands have been separated from Sulawesi since they first rose from the deep surrounding seas (Nugraha and Hall 2018), though the waters between the Wakatobi islands themselves are shallower (Figure 5.1). Deep, permanent water barriers are a key factor in the genetic isolation that leads to the evolution of new species (Wallace 1887), and several endemic bird taxa have been named from the Wakatobi (Hartert 1903, Collar and Marsden 2014, Kelly *et al.* 2014, O’Connell *et al.* 2019c). The Wakatobi, being islands of varying shapes, sizes, and distances from one another, thus provide an adequate context for testing the relationship between geography and genetic divergence, including the effects of island area, island elevation, and isolation by distance, following the approach of Linck *et al.* (2016). The four main islands of Wangi-Wangi, Kaledupa, Tomia and Binongko (whence the name “WaKaToBi”) all have maximum elevations over 200m and areas 53-155 km<sup>2</sup>, while Lintea Selatan and Hoga are just 8.3 km<sup>2</sup> and 3.5 km<sup>2</sup>, respectively, and are less than 20m above sea level. Menui is 183km from the Wakatobi.

Across the wide distribution of Island Monarchs, we hypothesised that genetic divergence would align with one of three general patterns:

**Pattern 1.** No divergence or limited divergence among populations across the entire species range, offering evidence of exceptional dispersal abilities that facilitate gene flow all the way from Melanesia to Wallacea.

**Pattern 2.** Two homogeneous sets of populations, one in Wallacea and one in Melanesia, divergent from one another but with little or no divergence within each set.

**Pattern 3.** Population structure within either Melanesia or Wallacea, or both, as well as genetic divergence between the two regions. This would indicate that the monarchs are not maintaining their dispersiveness after colonising these small islands, in turn suggesting that dispersiveness might not have been a selective response to these disturbance-prone conditions.

Divergence Pattern 1 would make the Island Monarch an example of “high dispersal facilities leading to a continuous swamping of semi-isolated populations” (Mayr 1942). Either of Patterns 1 or 2 would be in keeping with the original Diamond (1974) description of an archetypal supertramp. Pattern 3 would support an alternative conception, developed from genetic analyses of other supertramp taxa (Linck *et al.* 2016, Pepke *et al.* 2019), which sees the supertramps not as *r*-selected dispersal specialists, but as incipient species which will lose their dispersal abilities and form new island endemics. This developed from previous work on another class of highly dispersive birds, the “great speciators” (Moyle *et al.* 2009). In Divergence Pattern 3, supertramp status is not a permanent strategy but could instead be an early stage of the “taxon cycle” (Wilson 1959, 1961, Ricklefs and Bermingham 2002) of colonisation followed by range contraction (Pepke *et al.* 2019). The wider *r*- / *K*-selection hypothesis on which the supertramp theory was based has also evolved: where MacArthur and Wilson (1967) popularised a continuum of *r* selection in disturbance-prone environments to *K* selection in stable ones, this has since developed into a broader understanding of life-history strategies influenced by multiple factors (e.g. Reznick *et al.* 2002).

As the Pale-blue Monarch is endemic to land-bridge islands in Wallacea, any genetic structure across this range would characterise it as a markedly non-dispersive bird. The Southeast Sulawesi land-bridge islands were connected by land relatively recently in geological history (Nugraha and Hall 2018), and most birds have uniform populations across all of these islands (O’Connell *et al.* 2019a, O’Connell *et al.* 2019c), but there are exceptions (Robinson-Dean *et al.* 2002, Ó Marcaigh *et al.* 2021a, Ó Marcaigh *et al.* 2021b). Thus, strong genetic structure across its range in Wallacea would indicate that this sedentary, *K*-selected monarch, which appears to exclude the Island Monarch from these larger islands, has been subjected to strong selection against dispersal. This would support the broader theory around supertramp taxa. In contrast, an unstructured population would provide no evidence for a connection between competitive ability and dispersiveness. The inclusion of the Pale-blue Monarch also serves as a “control” for the confounding effects of mischaracterized geologic history in our “natural experiment”.

We present here an exploration of the population structure of the supertramp Island Monarch across both Wallacea and Melanesia, and that of the related, sedentary Pale-blue Monarch endemic to Wallacea. If the supertramp strategy is a product of *r* selection on small islands, then it should be reinforced with time in these habitats, resulting in an unstructured population. In contrast, structure in the Island

Monarch population would add more support to the supertramp strategy being a transient stage of the taxon cycle. Further, Linck *et al.* (2016) attributed the divergence of supertramps on larger and more elevated islands to selection against dispersal ability in these more stable habitats, but did not have morphological data to assess this directly. We have used morphologically-derived dispersal indices to investigate this hypothesis further. If mechanisms similar to those described in the Louisiade white-eye by Linck *et al.* (2016) were in effect, we predicted that larger, higher, and more isolated islands would hold the most genetically distinct populations. If this is driven by selection against dispersal ability, as could be expected under a relative  $K$  selection regime on larger and more permanent islands, we should also find populations on these islands to exhibit reduced dispersal ability. If they do not, this would imply a different relationship between dispersal, divergence and geography.

## 5.3 Methods

### 5.3.1 Sampling and DNA sequencing

We sampled birds by mist-netting on expeditions between 1999 and 2017 across Southeast Sulawesi, its nearby land-bridge islands, and the Wakatobi archipelago (Figure 5.1, Table 5.1). Birds were measured and photographed per Redfern and Clark (2001), to collect data on wing length (maximum chord) and body mass (in grams). We collected a small number of contour feathers from the flank of each bird, to allow extraction of DNA while minimising risk of injury and avoiding disruption to flight abilities and plumage-based visual signals (McDonald and Griffith 2011).

**Table 5.1.** Sample sizes for monarch populations included in study. A full list of birds used in analyses is available in the Supplementary Material (Table S5.1).

Species	Island	Archipelago	Region	Genetic Sample Size	Morphological Sample Size
<i>Hypothymis puella</i>	Buton	Sulawesi	Wallacea	2	5
<i>Hypothymis puella</i>	Kabaena	Sulawesi	Wallacea	3	4
<i>Hypothymis puella</i>	Muna	Sulawesi	Wallacea	3	3
<i>Hypothymis puella</i>	Southeast Sulawesi	Sulawesi	Wallacea	6	11
<i>Monarcha cinerascens</i>	Menui	Sulawesi	Wallacea	9	4

<i>Monarcha cinerascens</i>	Binongko	Wakatobi	Wallacea	5	5
<i>Monarcha cinerascens</i>	Hoga	Wakatobi	Wallacea	4	4
<i>Monarcha cinerascens</i>	Kaledupa	Wakatobi	Wallacea	6	19
<i>Monarcha cinerascens</i>	Lintea Selatan	Wakatobi	Wallacea	2	1
<i>Monarcha cinerascens</i>	Runduma	Wakatobi	Wallacea	4	3
<i>Monarcha cinerascens</i>	Tomia	Wakatobi	Wallacea	5	13
<i>Monarcha cinerascens</i>	Wangi-Wangi	Wakatobi	Wallacea	7	33
<i>Monarcha cinerascens</i>	Djaul	Bismarck	Melanesia	1	-
<i>Monarcha cinerascens</i>	Karkar	Bismarck	Melanesia	1	-
<i>Monarcha cinerascens</i>	Nusalaman	Bismarck	Melanesia	1	-
<i>Monarcha cinerascens</i>	Sulei	Solomon	Melanesia	1	-

We extracted DNA from feather samples using Qiagen DNeasy Blood and Tissue Kits. We followed the manufacturer's instructions but added 5µl of 1M dithiothreitol (DTT) at the digest phase to release any genetic material shielded by keratin in the feather calamus. We carried out Polymerase Chain Reactions (PCRs) to target the mitochondrial ND2 and ND3 genes using a touchdown cycling protocol to increase yield (Korbie and Mattick 2008), beginning the reaction with an annealing temperature 10°C hotter than the melting temperatures of the primers and gradually reducing it (reagent quantities and protocol provided in Figure S5.1). Evolutionary histories inferred from mtDNA sometimes differ from those inferred from nuclear DNA (Rubinoff and Holland 2005, Phillimore *et al.* 2008), and introgression and male-mediated gene flow can obfuscate biogeographic patterns (Toews and Brelsford 2012). However, in recent radiations ND2 and ND3 have been found to provide the best phylogenetic resolution (Andersen *et al.* 2015b), and mitochondrial DNA's sensitivity to population structure has proven useful in studies on biogeographic concepts like the supertramp strategy (Linck *et al.* 2016). As we aimed to study divergence and structure at the level of populations, we therefore deemed ND2 and ND3 to be appropriate markers for our ends. We targeted the ND3 gene using the L10755-F and DOC-ND3-R1 primer pair (Chesser 1999, O'Connell *et al.* 2019b) and amplified ND2 in two halves using internal and external primers of our own design (Figure S5.2, Table S5.2). We screened our PCR products using 2% electrophoresis gels stained with GelRed (Biotium) at a 1X

concentration, then had them Sanger sequenced by GATC EuroFins. We used the ClustalW function in BioEdit version 7.2.5 to align our sequences (Hall 1999) and concatenated the ND2 and ND3 sequences using Mesquite version 3.51 (Maddison and Maddison 2018), for a total of 1392 base pairs of DNA from each bird.

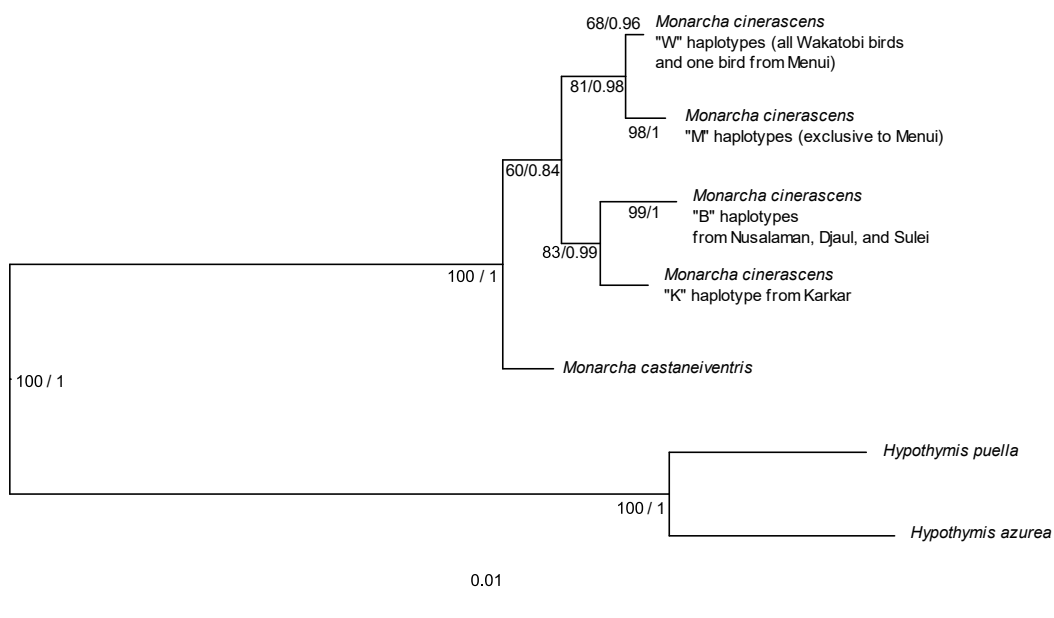
We used Island Monarch sequences from Andersen *et al.* (2015a) to represent the Melanesian population in our analyses. These represent three subspecies: *M. c. perpallidus* from Nusalaman and Djaul in the Bismarck Archipelago, *M. c. nigrirostris* from Karkar, and *M. c. impediens* from Sulei in the Solomon Islands. However, the taxonomy of this species is “complex and not fully understood”, and there is a proposal to lump these subspecies together (Billerman *et al.* 2022). We also utilised ten outgroup species from Andersen *et al.* (2015a), along with *Monarcha castaneiventris* sequences from Nyári *et al.* (2009a). A complete list of samples used in our analyses is available in the Supporting Information (Table S5.1).

### 5.3.2 Genetic analyses

We used both Maximum Likelihood (ML) and Bayesian approaches to investigate the phylogenetics of monarchs. Only one representative of each concatenated ND2/ND3 haplotype was included in the phylogenies. We used MEGA X version 10.1.8 (Kumar *et al.* 2018) to calculate the pairwise p-distances between these haplotypes (Tables S5.3 and S5.4). A p-distance is the proportion of sites that are different between the two sequences, uncorrected for multiple substitutions. We used the ModelFinder function (Kalyaanamoorthy *et al.* 2017) in IQTree version 1.6.12 (Nguyen *et al.* 2015) to select the most appropriate nucleotide substitution model for our alignment, which included *Monarcha* and *Hypothymis* haplotypes and an outgroup. Our ModelFinder analysis found strong support for a General Time Reversible model with a gamma shape parameter and a proportion of invariable sites (GTR+F+I+G4), supported by lower Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) than other models.

We conducted our ML analysis in IQTree using a GTR+F+I+G4 model. The confidence interval of the ML phylogeny was based on 1000 bootstraps (Felsenstein 1985). We used MrBayes version 3.2.7 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) to carry out Bayesian phylogenetic inference using a GTR+G+I model. This comprised two independent Markov chain Monte Carlo (MCMC) runs, with four chains per run, sampling every 1000 generations. We discarded the first 25% of each MCMC run as a “burn-in” (Mau *et al.* 1999). We assessed convergence in TRACER version 1.7.1 (Rambaut *et al.* 2018), deeming it acceptable once average standard deviation in split frequencies (ASDSF) reached 0.01 and

Effective Sample Size (ESS) of model parameters reached 200 (O'Connell *et al.* 2019c). Both of these requirements had been met by the time our MCMC algorithm reached 4 million generations. We exported a 50% majority rule consensus tree from MrBayes and imported both this and the ML tree into the R package “ggtree” (Yu *et al.* 2016). We produced a combined, simplified tree by omitting the outgroup and collapsing each major clade into a single branch, labelling the nodes with both ML bootstrap scores and Bayesian probabilities (Figure 5.2). We also made subtrees from the Bayesian tree, to display all haplotypes from *Monarcha* (Figure 5.3c) and *Hypothymis* (Figure 5.4c). Full versions of the Bayesian and ML trees, including all outgroup taxa, are provided in the Supplementary Material (Figures S5.3 and S5.4).



**Figure 5.2.** Simplified version of combined Maximum Likelihood (ML) and Bayesian phylogenetic tree of monarch species sampled in Wallacea and Melanesia. In this figure the outgroup is omitted and each major clade in the data is collapsed into a single branch. Nodes are labelled with ML bootstraps / Bayesian probability. Figures 5.3 and 5.4 include subtrees of the *Monarcha* and *Hypothymis* sections of the Bayesian tree, with individual haplotypes displayed. Full versions of the ML and Bayesian trees, including all outgroup taxa, are in the Supplementary Material (Figures S5.3 and S5.4).

We carried out distance-based molecular species delimitation on *Monarcha* and *Hypothymis* haplotypes using Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.* 2012). This method uses pairwise genetic distances to group haplotypes into “species” so that there are smaller genetic distances

within these “species” than between them. It takes a range of prior maximum intraspecific divergences, and for each of them it calculates a minimum threshold interspecific distance, the “barcode gap”. It then splits the haplotypes into groups, with the distance between each group equalling or surpassing the barcode gap. The range of prior maximum intraspecific divergences allows the user to calibrate the species groupings for different genes and species. We ran our ABGD analysis on the web-server <https://bioinfo.mnhn.fr/abi/public/abgd/> using default settings (Pmin = 0.001, Pmax = 0.1, Steps = 10, relative gap width = 1.5, Number of bins = 20) and a Kimura-2-Parameter (K2P) model.

To visualise any potential genetic structure in our monarch populations, we used PopArt version 1.7 (Leigh and Bryant 2015) to create TCS haplotype networks (Figures 5.3b and 5.4b). The TCS algorithm is agglomerative, progressively combining clusters that share one or more connecting edge (Templeton *et al.* 1992). This makes it well-suited to the analysis of divergence at the population level, where ancestral haplotypes are likely to be common in the population and variation is relatively low (Clement *et al.* 2000).

### 5.3.3 Morphological analyses

Classic supertramp theory describes certain species colonising distant islands due to superior dispersal abilities, but the “taxon cycle” hypothesis predicts they will lose these abilities and become differentiated (Pepke *et al.* 2019). We tested this using morphological analyses on adult Island Monarchs from Wallacea, carried out in R version 4.0.2 (R Core Team 2021). These analyses investigated whether genetic divergence of populations was accompanied by a reduction in dispersal ability. Four adults were sampled in Menui, three of them processed by a different recorder (DOC) than the other birds in our dataset (which were processed by NMM). To test for differences between the measurements of DOC and NMM, five birds of various species were measured by both authors (Table 5.2). We ran a paired 2-sample *t*-test on their measurement data to check for inconsistent measurements.

**Table 5.2.** Birds measured by both DOC and NMM for comparison of ringers.

ID	Species	Island	Wing length measured by NMM (mm)	Wing length measured by DOC (mm)
SUL2285	<i>Zosterops consobrinorum</i>	Muna	55	56
SUL2361	<i>Zosterops consobrinorum</i>	Muna	58	58.5

<b>SUL2564</b>	<i>Collocalia esculenta</i>	Menui	100	100
<b>SUL2573</b>	<i>Leptocoma aspasia</i>	Menui	59	60
<b>SUL2581</b>	<i>Monarcha cinerascens</i>	Menui	76	76

Wing length alone is a poor indicator of dispersal ability (Dawideit *et al.* 2009). We therefore followed Garrard *et al.* (2012) and O’Connell *et al.* (2019c) by using our morphological data to derive an allometrically scaled “shape” parameter termed the “dispersal index”:

$$\text{Dispersal index} = (\text{wingspan})^3 / \text{body mass}$$

We calculated wingspan from wing length using the formula from Garrard *et al.* (2012), devised from species for which both wingspan and wing length were known:

$$\text{Wingspan} = 1.91 (\text{wing length}) + 0.06$$

Because of the physical constraints of flight, wingspan is expected to increase by three units whenever body mass increases by one unit (Garrard *et al.* 2012). The dispersal index is thus designed to be informative of variation in wingspan beyond that resulting from variation in body mass. We ran a *t*-test to compare the dispersal indices of the two clades suggested by the genetic analyses for which we had morphological data, and incorporated the dispersal index into our biogeographic analyses.

### 5.3.4 Biogeographic analyses

Following Linck *et al.* (2016), we ran three linear models to test the effect of different biogeographic considerations on genetic divergence, measured as the mean p-distance between that island’s monarchs and those of its closest neighbour. Model A was designed to test the effect of an island’s area on genetic divergence, Model B the effect of island elevation, and Model C the effect of the island’s geographic isolation (distance from the closest neighbouring population). Linck *et al.* (2016) described Models A and B as supertramp-specific, with a prediction of positive correlations between area/elevation and genetic divergence due to supertramps losing their dispersal abilities on larger and higher islands. Area and elevation are classic biogeographic proxies for the permanence of an island over geological time (MacArthur and Wilson 1967, Mayr and Diamond 2001). Model C is designed to test for more general “isolation by distance” (Wright 1943).



Our sampling allowed us to replicate these models using both Wallacean and Melanesian islands. We used NaturalEarth polygons and the R package “geodist” (Padgham *et al.* 2021) to find the closest points of islands and measure the distance between them. We collected data on area and elevation from the literature (Beehler and Pratt 2016, Monkhouse *et al.* 2018, O’Connell *et al.* 2019a, Kumar 2020). Because the Melanesian sampling of Andersen *et al.* (2015a) did not cover the Eastern Bismarcks or Western Solomons, the Sulei population had an artificially inflated geographic distance to its “nearest neighbour”, Djaul. Our Model C therefore used the logarithm of geographic isolation in order to better reflect the true variation in isolation. We compared models using base 2, base 10, and the natural log, and with the raw geographic isolation data with and without Melanesian data.

We also developed four additional biogeographic models that incorporated our Island Monarch morphology data, to test the conclusion of Linck *et al.* (2016) that supertramp species become differentiated on larger and more permanent islands due to selection against dispersal ability. These, Models W, X, Y, and Z, only covered Wallacea as we had no morphological data from Melanesia. Model W tested the relationship between genetic divergence, measured as the mean p-distance between that island’s monarchs and those of its closest neighbour, and dispersal ability, i.e. the mean dispersal index of the island’s monarchs. Model X tested the relationship between geographic isolation and dispersal ability. Model Y tested dispersal ability’s relationship with island area, Model Z its relationship with island elevation. We drew lines of best fit to visualise our models using the packages “ggplot2” (Wickham 2016) and “ggrepel” (Slowikowski 2021).

## 5.4 Results

### 5.4.1 Genetic results

In total we produced 42 new concatenated ND2-ND3 sequences for the Island Monarch and 14 for the Pale-blue Monarch (overview in Table 5.1, full list in Table S5.2). We have deposited these in GenBank (accession numbers MZ604441– MZ604552).

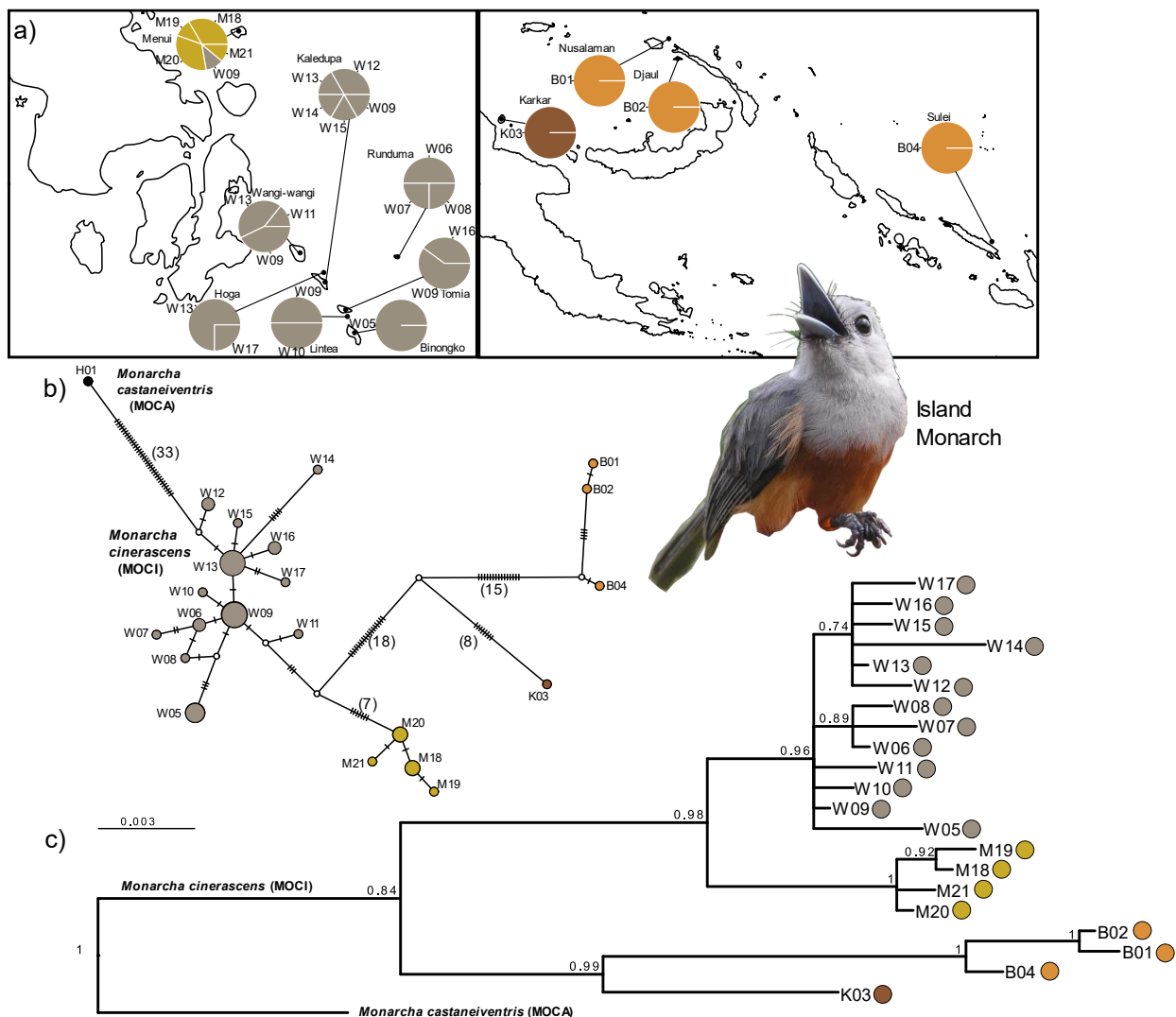
Bayesian and ML phylogenetic reconstruction sorted the Island Monarchs into four main clades (Figure 5.2), with geographic structure evident both from the trees and genetic p-distances. The mean genetic distance between Wallacea and Melanesia was 2.7% and there were also divisions within each of these regions. Clade W included all the birds netted on the Wakatobi islands and one immature bird netted

on Menui. The bootstrap value supporting Clade W was 68, but the Bayesian probability was robust at 0.96. The other eight birds netted on Menui made up a separate clade, M, supported by a strong bootstrap value of 98 and a Bayesian probability of 1. The mean distance between the M and W clades was 1%. The branch lengths (Figure 5.3) also support strong divergence between clades W and M. In Melanesia, the bird from Sulei in the Solomon Islands grouped with the two birds from the central Bismarcks (Djaul and Nusalaman) in a well-supported Clade B, which had a bootstrap value of 99 and a Bayesian probability of 1. The Karkar bird had a distinct haplotype (MOCI\_K03) with a mean distance of 1.9% from Clade B. Within-group variability was 0.2% in Clade W, 0.08% in Clade M, and 0.3% in Clade B. Branch support for monophyletic *M. cinerascens* was comparatively low (bootstrap value of 60, Bayesian probability of 0.84).

The genetic distance we found between Island Monarchs of Wallacea and Melanesia (2.7%) is closely equivalent to that between well-differentiated monarch species, as *Monarcha castaneiventris* (MOCA\_H01) had a mean distance of 2.6% from our Wallacean samples and 2.8% from the Melanesian birds. The ABGD analysis separated our Island Monarch haplotypes into either three or four molecular “species” depending on the prior intraspecific divergence. At the more conservative end, it calculated 1.6% as the barcode gap distance, which is in line with interspecific ND2 barcode gaps found by other studies on birds (Pellegrino *et al.* 2017, O’Connell *et al.* 2019c), and with this it divided the haplotypes into three groups. Pairwise distances between all haplotypes are available in the Supplementary Material (Table S5.4).

We found that the patterns of pairwise p-distances between islands and ABGD analyses of Island Monarch haplotypes aligned with geographic divisions (Figure 5.3). At levels of interspecific divergence similar to those seen in other birds (Pellegrino *et al.* 2017, O’Connell *et al.* 2019c), ABGD divided the Island Monarch into three species: one on Karkar (MOCI\_K03), one on the other Bismarck and Solomon islands (MOCI\_B01, B02, and B04), and one in Wallacea (MOCI\_W05-M21). Species-level divergence between Wallacea and Melanesia is well-supported, matching the divergence between *M. cinerascens* and *M. castaneiventris*. Additionally, there is evidence of weaker divergence (likely below species level) within the Wallacean birds. With a smaller barcode gap of 0.7%, the ABGD produces a fourth group composed of haplotypes found exclusively in birds from Menui (MOCI\_M18-21). These Menui-exclusive haplotypes represent eight of the nine birds caught on Menui (Figure 5.3a). One bird from Menui carried a haplotype (MOCI\_W09) common to four of the Wakatobi islands. The (W)akatobi, (M)enui, (K)arkar, and (B)ismarck groups can all be seen as distinct clusters on the haplotype network (Figure 5.3b) and monophyletic groupings on the phylogenetic tree (Figure 5.3c). Together, this evidence supported Divergence Pattern 3: a

species-level division between Wallacean and Melanesian populations, and population structure within both regions.



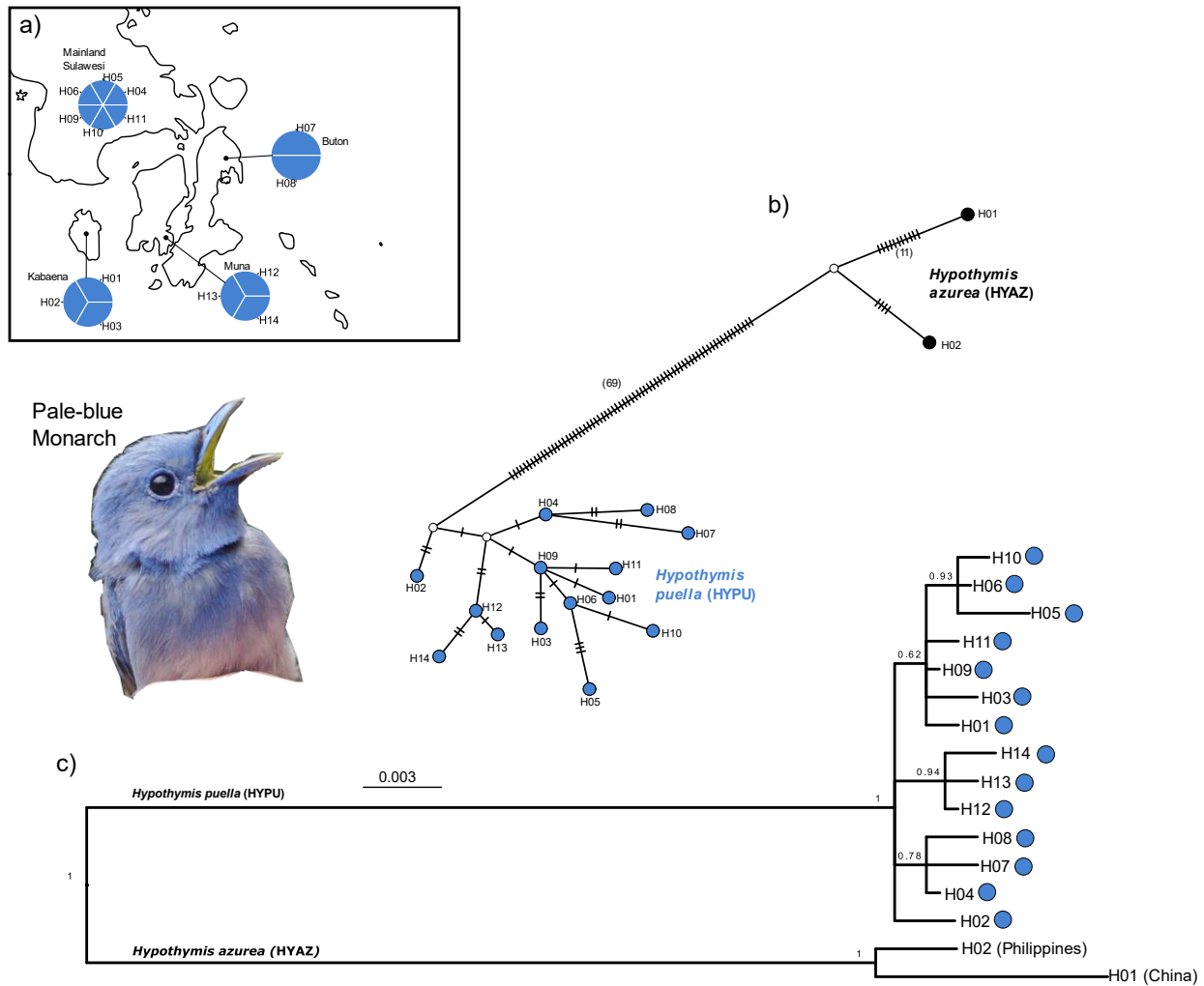
**Figure 5.3.**

**a)** Geographic distribution of Island Monarch (*Monarcha cinerascens*) haplotypes in Wallacea and Melanesia. Each circle represents an island and the fractions within the circle the haplotypes found in that region, with the size of each proportion representing the frequency of each haplotype. The haplotypes are named and coloured according to clade.

**b)** TCS Haplotype Network of *Monarcha* haplotypes. Each circle represents a unique ND2-ND3 haplotype, named and coloured according to clade and sized to represent how many birds carried that haplotype. The hatch marks represent mutations between haplotypes, also given as numbers in brackets for the wider divergences. The unfilled, white nodes represent hypothetical ancestral states.

**c)** Bayesian consensus tree of *Monarcha* haplotypes. Nodes are labelled with Bayesian probabilities.

Between the islands inhabited by the Pale-blue Monarch, which were connected by land bridges in recent geological history, there was no geographic population structure evident (Figure 5.4), and no two haplotypes of this species were more than 0.6% different from one another (Table S5.3).



**Figure 5.4.**

**a)** Geographic distribution of Pale-blue Monarch (*Hypothymis puella*) haplotypes in Wallacea. Each circle represents an island and the fractions within the circle the haplotypes found in that region, with the size of each proportion representing the frequency of each haplotype. All haplotypes are shown with the same letter (H) and colour (blue) because no population structure was found.

**b)** TCS Haplotype Network of *Hypothymis* haplotypes. Each circle represents a unique ND2-ND3 haplotype, sized to represent how many birds carried that haplotype. The hatch marks represent mutations between haplotypes, also given as numbers in brackets for the wider divergences. The unfilled, white nodes represent hypothetical ancestral states.

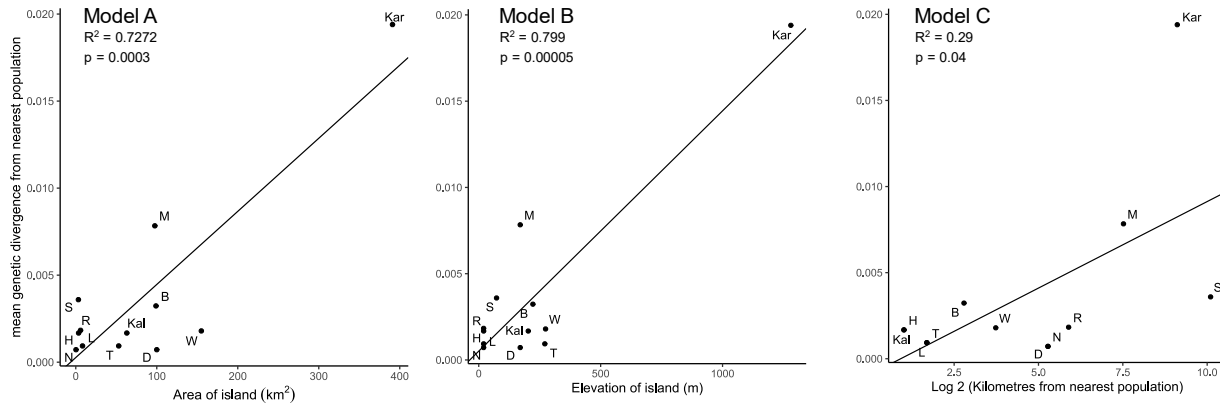
**c)** Bayesian consensus tree of *Hypothymis* haplotypes. Nodes are labelled with Bayesian probabilities.

### 5.4.2 Morphological results

Of the five birds measured in order to compare measurements by both DOC and NMM (Table 5.2), the same wing length was recorded in two cases (including the only Island Monarch measured by both recorders), while DOC measured the wing length as 0.5-1mm longer than NMM in the others. Our paired two-sample *t*-test found a mean difference of 0.5 between the two recorders, which was not statistically significant ( $p = 0.08$ ). This allowed us to include both recorders' data. Bird SUL2219, the one Island Monarch caught on Menui with a Wakatobi haplotype (MOCI\_W09), had to be excluded from morphological analyses as it was immature. The M clade has a higher dispersal index than the W clade (*t*-test,  $p < 0.0001$ ).

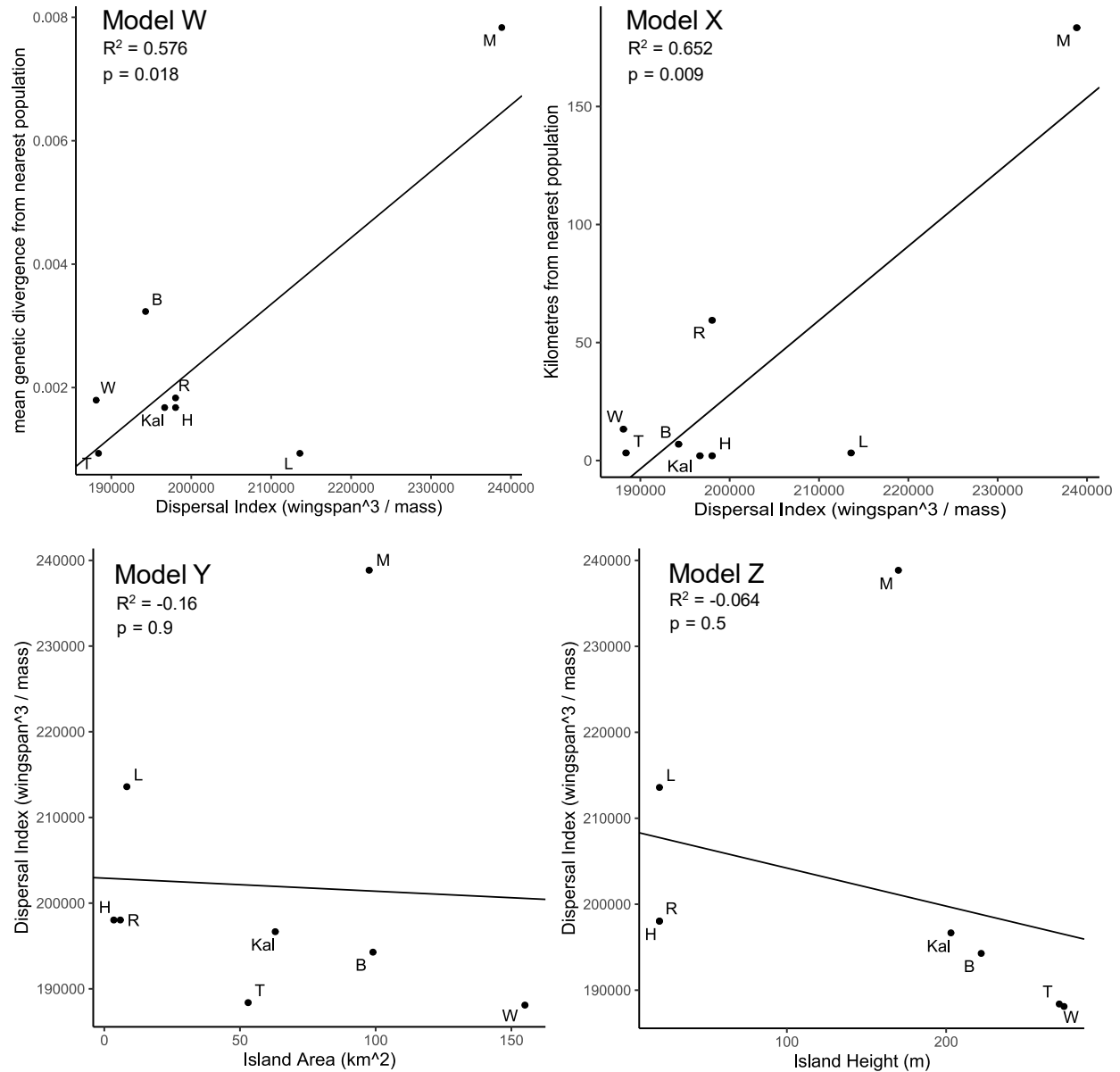
### 5.4.3 Biogeographic results

We found support in our data for the three models (Figure 5.5) proposed by Linck *et al.* (2016). There was a strong positive correlation between the area of an island and the genetic divergence of its Island Monarchs (Model A,  $p < 0.001$ ,  $R^2 = 0.73$ ). There was also a positive relationship between the elevation of an island and the genetic divergence of its monarch population (Model B,  $p < 0.0001$ ,  $R^2 = 0.8$ ). Like Linck *et al.* (2016), we found a relatively weaker positive relationship between genetic divergence and geographic isolation. While this relationship was statistically significant, it explained less of the variation (Model C,  $p < 0.05$ ,  $R^2 = 0.29$ ). This was consistent whether Model C used the logarithm in base 2, base 10, or the natural log of geographic isolation. When not using a log scale, Model C found a relationship in Wallacea, where geographic coverage was complete ( $p < 0.01$ ), but not when including Melanesia, where the sampling was incomplete ( $p > 0.1$ ). This supports the hypothesis that the gap in sampling between Sulei and Djaul in Melanesia turns Sulei into an outlier artificially, and that the log scale counters this outlier.



**Figure 5.5.** Linear regressions of genetic divergence between neighbouring Island Monarch populations fit to three island biogeographic models. These are genetic divergence by (A) island area, (B) island elevation, and (C) geographic isolation. Genetic divergence is the mean proportion of varying nucleotides (uncorrected p-distance) in concatenated ND2-ND3 sequences. Each data point represents an island in Wallacea or Melanesia: (M)enui, (W)angi-Wangi, (Kal)edupa, (T)omia, (B)inongko, (H)oga, (R)unduma, (L)intea Selatan, (N)usalaman, (D)jaul, (S)ulei, and (Kar)kar.

To these we added models (Figure 5.6) exploring the relationship between dispersal ability and both genetic differentiation and island parameters in Wallacea. We found dispersal ability to be related positively to both genetic divergence (Model W,  $p < 0.05$ ,  $R^2 = 0.58$ ) and geographic isolation (Model X,  $p < 0.01$ ,  $R^2 = 0.65$ ). But we found no correlation between dispersal ability and island size (Model Y,  $p = 0.5$ ) or island elevation (Model Z,  $p = 0.48$ ).



**Figure 5.6.** Linear regressions of genetic divergence and dispersal ability of Island Monarch populations fit to four island biogeographic models. These are W) genetic divergence as a function of dispersal ability, X) dispersal ability as a function of geographic isolation, Y) dispersal ability as a function of island area, and Z) dispersal ability as a function of island elevation (permanence). Genetic divergence is the mean proportion of varying nucleotides (uncorrected p-distance) in concatenated ND2-ND3 sequences. Each data point represents an island in Wallacea: (M)enui, (W)angi-Wangi, (Kal)edupa, (T)omia, (B)inongko, (H)oga, (R)unduma, (L)intea Selatan.

## 5.5 Discussion

### 5.5.1 Phylogenetics and biogeography

Islands in the “natural laboratory” of Wallacea (Whittaker *et al.* 2017), where the fauna of Asia transitions into that of Australo-Papua and the Pacific (Ali and Heaney 2021), have been colonised by monarch flycatchers (Monarchidae) with contrasting life history strategies. The Pale-blue Monarch is a sedentary resident species from an Asian lineage, while the Island Monarch is one of Melanesia’s original supertramps (Diamond 1974). Despite their different origins and inferred differences in dispersal ability, we find that the natural experiment has resulted in both island and Pale-blue Monarchs diverging significantly from their closest relatives outside Wallacea, implying that the deep water barriers around this region have cut off gene flow from outside.

The Pale-blue Monarch’s split from the Black-naped Monarch demonstrates that even a bird with a range from India to Borneo has difficulty crossing to Sulawesi across a deep water barrier. Within the Pale-blue Monarch, however, we have found no population structure at the scale of this study (Figure 5.4). The Pale-blue Monarch shares this pattern with the region’s relatively dispersive birds like the white-eyes (O’Connell *et al.* 2019c) and sunbirds (O’Connell *et al.* 2019a, see also Chapter 6 of this thesis), a pattern that contrasts with non-dispersive birds like the Red-backed Thrush (Robinson-Dean *et al.* 2002) and Sulawesi Babbler (Ó Marcaigh *et al.* 2021b), which do exhibit divergence on this same scale. Thus the Pale-blue Monarch does not provide evidence for a link between competition, *r*-/*K*-selection, and dispersiveness.

In the Island Monarch, we found that Melanesian and Wallacean birds were as genetically distant from one another (mean 2.7%) as they were from the unambiguously different species *M. castaneiventris* (2.8% and 2.6% mean distance from Melanesia and Wallacea, respectively). We also identified population structure within both Wallacea and Melanesia, supporting Divergence Pattern 3. In Melanesia, ABGD identified the Karkar population (a single sample) as a potential species. Though Karkar is geologically part of the Bismarck volcanic arc (Silver *et al.* 2009), its proximity to the New Guinea mainland has a strong influence on its native fauna, to the point that biogeographers consider it part of the New Guinea region rather than the Bismarck region (Mayr 1941, Beehler and Pratt 2016). Diamond and Lecroy (1979) noted the peculiarity of Karkar’s birds, describing it and neighbouring Bagabag Island as “a zoogeographically interesting mixing zone” between New Guinea and the Bismarck archipelago. Though the Solomon Islands



are quite distant from the Bismarcks and their monarchs are named as a separate subspecies (*Monarcha cinerascens impediens*), our ABGD analysis assigned the Sulei haplotype to the same population as haplotypes from Nusalaman and Djaul in the Bismarcks. This may support the hypothesis of Mayr and Diamond (2001) that the species originated in the Bismarcks and only colonised the Solomons recently.

A Menui population was found to be distinct based on a larger sample size. Of the nine birds netted on Menui, eight of them (88.9%) formed “Clade M”, with haplotypes unique to Menui and a mean distance of 1% from the other Wallacean birds in Clade W (Figure 5.3). These birds also exhibited higher dispersal indices than those from the Wakatobi. Menui is 183km from the Wakatobi, a relatively short distance for a supposed dispersal specialist which, according to current taxonomy, maintains a range across more than 4000 kilometres of deep water. That the Island Monarch population of Menui remained undocumented until so recently (Monkhouse *et al.* 2018) demonstrates how our lack of knowledge of little-explored areas of bird endemism still hampers our understanding (Rheindt *et al.* 2020). Inadequate species distribution data (“Wallacean shortfalls”) often complicate conservation efforts (Whittaker *et al.* 2005). One immature bird netted on Menui (ID SUL2547) carried a “W” haplotype common to the Wakatobi islands (MOCI\_W09), also found in birds from Wangi-Wangi, Lintea Selatan, Hoga, and Tomia (Figure 5.3). This may be evidence of natal dispersal from the Wakatobi to Menui, without enough gene flow to prevent genetic divergence, in keeping with the “occasional wandering” of Island Monarchs from one differentiated population to another described by Mayr (1944). Alternatively, it may not have been a natural movement: birds are regularly translocated around Indonesia in a highly active bird trade, giving rise to an “Asian Songbird Crisis” (Marshall *et al.* 2020). Birds from Wangi-Wangi are known targets (O’Connell *et al.* 2021). This trade has existed for centuries and even Wallace’s biogeographic studies were sometimes complicated by it, as he noted of the region’s parrots that “The greatest confusion exists as to their distribution, owing to their being carried from island to island by the native traders” (Sclater 1859).

### 5.5.2 Implications for the supertramp strategy

Evolutionary biology and biogeography owe many of their most important insights to the diversity and distribution patterns of island bird communities (Darwin 1859, Wallace 1863, MacArthur and Wilson 1967, Mayr and Diamond 2001). Modern techniques, applied to previously little-explored areas, allow these foundational frameworks to be continually updated. Recent work on distributions (mapped in Eaton *et al.* 2021) has made it clear that the Island Monarch exhibits the same “supertramp” distribution in Wallacea as it

does in Melanesia, being restricted to small islands while the Pale-blue Monarch occupies the larger islands. This pattern of island occupancy is thus reinforced as a predictable phenomenon that warrants an ecological or evolutionary explanation. It may be the invasibility of communities present on larger islands, rather than the incidence of island arrival events alone, that explain the absence of the Island Monarch, but precise causes are far from being understood. Further, the Island Monarch has diverged most strongly on larger, higher, and more distant islands (Models A, B, and C), in keeping with predictions based on previous work with the supertramp Louisiade white-eye (Linck *et al.* 2016). The supertramp strategy was initially envisioned as an adaptive response to the *r*-selective environments of small islands (Diamond 1974). If such adaptation were ongoing, our study system of small islands would be subject to “continuous swamping”, and thus hold an unstructured population “without noticeable geographic variation” (Mayr 1942). Instead, we found substantial divergence between Wallacea and Melanesia, and lesser divergence within each region (Divergence Pattern 3). It is possible that what we call supertramp species simply correspond to lineages with high colonising potential, with island populations tending to differentiate as a result of a reduced propensity to disperse after they establish on islands.

Across the Wallacean transition zone and into Melanesia, the Island Monarch appears to be making a transition of its own: from a widespread supertramp to a number of differentiated populations, each of them effectively sedentary and resident in a small area. Our findings support those of Linck *et al.* (2016) in showing that another of the classic supertramp species exhibits population structure on a relatively fine geographic scale. Like Linck *et al.* (2016) and Pepke *et al.* (2019), our results indicate that the supertramp condition appears to be temporary, and the populations in question are in fact incipient species which will differentiate over time. Our findings support Pepke *et al.* (2019) in integrating the supertramp condition into the well-established concept of the taxon cycle (Wilson 1959, 1961). Originally devised for ants, this cycle describes a series of stages that taxa pass through as they expand their ranges by colonising islands, then evolve into differentiated lineages, then undergo range contraction. Based on our findings, this seems to describe the history of monarch flycatchers in these regions quite well, with different *Hypothymis* and *Monarcha* populations occupying different points on the cycle.

Our dispersal index analysis let us probe deeper and explicitly examine the biogeographic underpinnings of dispersal ability. Our findings in this regard were contrary to the prediction of Linck *et al.* (2016) that conditions on larger, more permanent islands would select against dispersal ability. Our data suggest that the most dispersive birds were able to colonise the most isolated islands (Model X), where they

diverged due to isolation by distance (Model C), as even their strong dispersal ability did not cause enough gene flow to prevent differentiation (Model W). Most divergence took place on larger and higher islands (Models A and B), but there is no evidence of reduction in dispersal ability under these stable conditions (Models Y and Z). In fact, it is to be expected that more permanent islands should hold the most differentiated populations, regardless of selective pressures. In their discussion of radiation, MacArthur and Wilson (1967) noted that more stable islands simply provide more time in which evolution can take place. Outside the supertramp debate, sedentary Wallacean residents like the *Ninox* owls have been shown to have diverged most on larger, higher islands (Gwee *et al.* 2017). Rather than losing their physical dispersal ability, the divergent Island Monarch populations could have evolved “behavioural flightlessness” (Diamond 1981, Komdeur *et al.* 2004, Bertrand *et al.* 2014). This is known to occur in other birds which are physically well adapted to dispersal, like *Zosterops* white-eyes (Moyle *et al.* 2009). Taken together, these studies suggest that similar processes govern genetic divergence in both highly dispersive and less dispersive species. In other words, between the speciation of supertramps and that of sedentary species there are differences “of degree and not of kind” (Darwin 1871).

Our findings illustrate that supertramp status can be viewed more accurately as an ecological rather than an evolutionary condition, or as the instigator of biogeographic patterns rather than the outcome. After colonising islands, the supertramp appears to “settle down” and transition to a resident species. The size, permanence, and isolation of the islands shape the population structure that emerges. Even if the supertramp lifestyle is a temporary phase within the taxon cycle, it is nevertheless an important factor in the region’s biodiversity as it regularly produces new island communities which seed new evolutionary processes. As our study sampled a single supertramp species, we recommend that more of the original supertramps should be studied genetically, as the Louisiade White-eye and now the Island Monarch have been. This will allow scientists to continue building a complete picture of this fascinating mosaic of distribution and divergence.

### 5.5.3 Taxonomy

Our work has several implications for taxonomy, but further work is needed before new taxa can be described. Based on the genetic divergence we have found, the “Island Monarch” studied by Mayr and Diamond (2001) in Melanesia may be a cryptic species absent from Wallacea. The Island Monarch was described by Temminck (1827) from a specimen collected on Timor in Wallacea, an island it is now known to

visit only in the monsoon (Eaton *et al.* 2021). Additional sampling in southern and eastern Wallacea, or sequencing of existing museum specimens, would therefore help to clarify whatever taxonomic divisions exist within the currently described Island Monarch. We also found variation within each region, divided by smaller genetic distances. Geographically circumscribed clades which are genetically and morphologically distinct, but are not reproductively isolated, may warrant subspecies status (Patten 2015). In Melanesia, the genetically distinct Island Monarchs of the Karkar area are already named as a subspecies, *Monarcha cinerascens nigrirostris* (Andersen *et al.* 2015a). In Wallacea, we recommend that more monarchs should be sampled on Menui and that the Banggai and Sula islands to its north (not sampled for this study) should be examined. These populations may reveal undocumented subspecies.

#### 5.5.4 Conclusion

As both Melanesia and Wallacea are of global concern for the conservation of biodiversity (Myers *et al.* 2000, Brooks *et al.* 2006), as well as being foundational to the history of biogeography (Wallace 1860, Mayr and Diamond 2001), it is vital that we understand where and how these regions' species are evolving. Better knowledge of their diversity, distributions, and evolutionary distinctness would allow conservationists to target the most relevant populations and biogeographers to fully develop the field's most influential theories.

Wallace (1860) noted that “however narrow may be the strait separating an island from its continent, it is still an impassable barrier against the passage of any considerable number and variety of land animals; and that in all cases in which such islands possess a tolerably rich and varied fauna of species mostly identical, or closely allied with those of the adjacent country, we are forced to the conclusion that a geologically recent disruption has taken place.” Our findings reinforce that this may even be true for dispersive birds: in both supertramp and sedentary monarchs, geologically recent land bridges allow islands to share populations, while even relatively narrow water barriers will cause genetic divergence as long as they are deep and, therefore, permanent. It is the number and diversity of such barriers around Wallacea that make it “a district *per se*, in the highest degree interesting” (Wallace 1860). Integrative taxonomy and comprehensive sampling are necessary to understand the diversity and distributions of species (Cicero *et al.* 2021) and, from there, the theoretical concepts these species have inspired.

## Chapter 6 - Small islands and large biogeographic barriers have driven contrasting speciation patterns in Indo-Pacific sunbirds (Aves: Nectariniidae)

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*“...But such a form as Grecian goldsmiths make  
Of hammered gold and gold enamelling  
To keep a drowsy Emperor awake;  
Or set upon a golden bough to sing  
To lords and ladies of Byzantium  
Of what is past, or passing, or to come.”*  
- W.B. Yeats (1928)

**Author Contribution:** I conceived this study with Darren O’Connell, David Kelly, and Nicola Marples. I wrote the manuscript with input from Nicola, David, and Darren. Nicola and David led the fieldwork, with contributions from Darren, Adi Karya, Kangkuso Analuddin, and me. I carried out the labwork, with assistance from Jennifer McCloughan and Naomi Lawless. I supervised Ellen Tolan in analysing the acoustic data for her undergraduate dissertation. I conducted the other data analyses and produced all of the figures.

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**Chapter Illustration:** Olive-backed Sunbird female (top) and male (bottom), from Shelley (1876-1880).

## 6.1 Summary

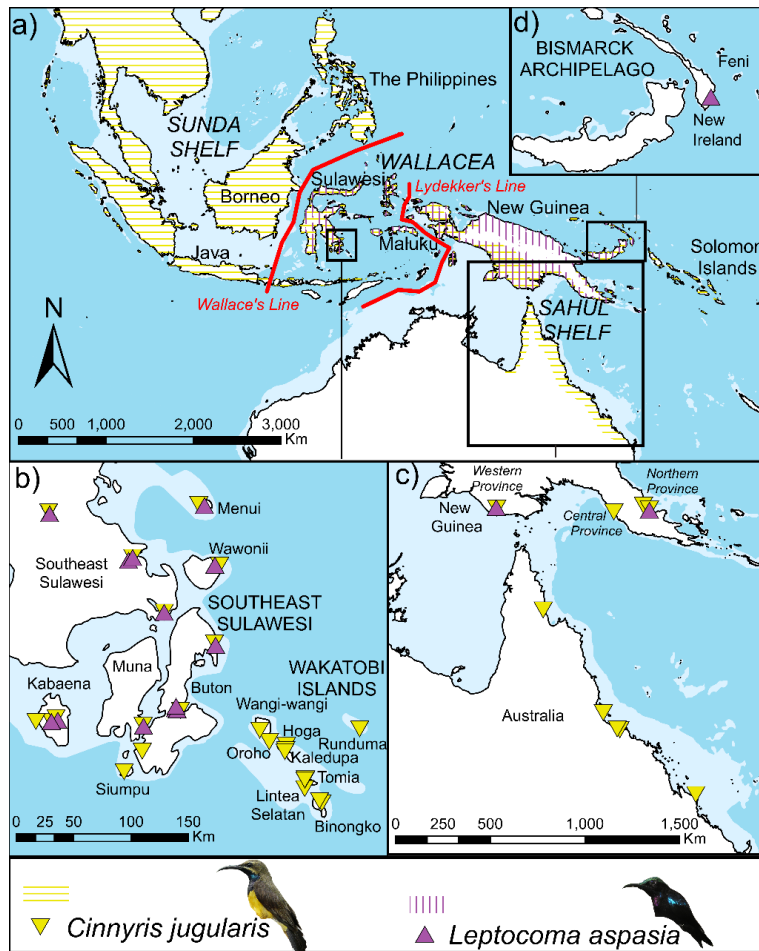
The birds of the Indo-Pacific have provided biologists with foundational insights. This study presents evidence for strong phylogeographic structure in two sunbird species from the heart of this region, the Olive-backed sunbird *Cinnyris jugularis* and the Black sunbird *Leptocoma aspasia*. We assessed population divergence using morphological, plumage, bioacoustic and molecular data (mitochondrial ND2/ND3). Our findings indicate that the Olive-backed Sunbird should be recognised as a complex of multiple species, as birds from Sulawesi and the Sahul Shelf are closely related to each other, but widely separated from those in other regions. In addition, we provide evidence for an endemic species on the Wakatobi Islands, an archipelago of deep-sea islands off Southeast Sulawesi. That a small bird could exhibit a range all the way from Sulawesi to Australia, while diverging on a small archipelago within this range, illustrates the complex interplay between dispersal and speciation. Our Black Sunbird genetic data also suggest unrecognised population structure, despite relatively weak plumage divergence. Black Sunbirds in Sulawesi are likely to be a separate species from those in New Guinea, with a mean genetic distance of 9.1%. Current taxonomy suggests these sunbird species transcend classic biogeographic barriers, but our results suggest that these barriers are not easily bypassed.

## 6.2 Introduction

### 6.2.1 Biogeography of Wallacea

Wallacea (Figure 6.1) is a region composed of islands separated by deep water in central Indonesia, situated between the much shallower Sunda and Sahul continental shelves (Merrill 1924, Dickerson *et al.* 1928). Due to changes in sea level during glaciations (Voris 2000), the boundaries between these contrasting water depths have acted as barriers to the dispersal of many organisms, leading to marked differences in the plants and animals found on either side (Lohman *et al.* 2011). Wallacean islands played a vital role in the evolution of songbirds, providing avenues for dispersal and radiation after the group originated in Australia (Moyle *et al.* 2016). Wallacea's largest island, Sulawesi has a complex geological history that has shaped its marked patterns of biological endemism (Michaux and Ung 2021). The western boundary between Wallacea and the Sunda Shelf is known as Wallace's Line (Wallace 1863, Huxley 1868), though Wallace had difficulty deciding where to position his line relative to Sulawesi (Ali and Heaney 2021) and considered this island "anomalous" (Wallace 1880). The eastern boundary between Wallacea and the Sahul Shelf was first described as a biogeographic barrier by Heilprin (1887) but is now best known as Lydekker's Line (Lydekker 1896, Ali and Heaney 2021). As a zone of transition between strikingly different biotas (Merrill 1924, Dickerson *et al.* 1928),

Wallace has furnished the field of biogeography with many foundational insights (Wallace 1860, 1863), and work in the region continues to improve our understanding of evolutionary theory in general as well as the evolutionary histories of many different organisms (Moyle *et al.* 2016, Rowe *et al.* 2019, Purnomo *et al.* 2021, Hardianto *et al.* 2022).



**Figure 6.1.**

**a)** Map of the Indo-Pacific region with focal study regions inside boxes. The range of the Olive-backed Sunbird is shaded horizontally in yellow, the range of the Black Sunbird vertically in purple, both according to BirdLife International. Seas deeper than 200 metres are represented by a darker blue. Biogeographic barriers (Wallace 1863, Lydekker 1896) are represented with red lines.

**b)** Map of Southeast Sulawesi and the Wakatobi Islands in Wallacea, with Olive-backed Sunbird sampling sites marked with yellow downward-pointing triangles, Black Sunbird sampling sites with purple upward-pointing triangles.

**c)** Map of Australia and New Guinea on the Sahul Shelf, with Olive-backed Sunbird sampling sites marked with yellow downward-pointing triangles, Black Sunbird sampling sites with purple upward-pointing triangles.

**d)** Map of the Bismarck Archipelago with the sampling site of the B10K Black Sunbird marked with a purple triangle.

Wallacea has been recognised for some time as a hotspot of threatened biodiversity (Myers *et al.* 2000). The importance of Wallacean biodiversity is becoming ever more apparent: the latest edition of the current reference work on the region's birds (Eaton *et al.* 2021) recognises 27 additional endemic species compared to the first edition, published just over four years earlier. Eaton *et al.* (2021) sorted their taxonomic recommendations into two categories: splits and "limbo splits", which are "possible splits that have either been mentioned in the literature but we feel that support is weak or insufficient, or they have generally not been mentioned in the previous literature and we feel that potential for splitting is considerable" (Rheindt 2021). The vast majority of new Wallacean taxa, including both splits and limbo splits, are restricted to particular islands (Eaton *et al.* 2021) and are thus strictly allopatric. The consistent delimitation of allopatric taxa remains challenging, even when data are available (Tobias *et al.* 2021). Therefore, specific and detailed investigation is still needed to clarify the diversity of birds across Wallacea's many islands. One solution to the problem of allopatry (e.g. Cheke *et al.* 2001, Mayr and Diamond 2001) is to deal with "superspecies", defined as monophyletic groups of allopatric populations that are inferred to be reproductively isolated, based on comparison with sympatric species (Amadon 1966).

Wallacea's islands are diverse in sizes and their degree of isolation, making Wallacea an ideal "natural laboratory" (Whittaker *et al.* 2017) for the study of biogeographic questions (O'Connell 2019, Ó Marcaigh *et al.* 2021a, Ó Marcaigh *et al.* 2021b, Ó Marcaigh *et al.* 2022b). For instance, in the Southeast Sulawesi region there are continental land-bridge islands like Wawonii (or Wowoni), Kabaena, Muna, and Buton (or Butung), which were connected by land to the much larger Sulawesi and to each other during geologically recent glaciations (Hall 2013). On the other hand, the smaller Wakatobi Islands (also known as the Tukangbesi Islands) have not been attached to any larger landmasses since they first emerged (Nugraha and Hall 2018). The Wakatobi Islands are recognised as an Important Bird Area and Key Biodiversity Area (BirdLife International 2021), but despite their importance they received little ornithological attention until recently (O'Connell *et al.* 2020b). Though the Wakatobi Islands are just 27km from Buton, they harbour several endemic species (Kelly *et al.* 2014, O'Connell *et al.* 2019c), evidence of significant evolutionary independence from Sulawesi and its land-bridge islands. An additional small island, Menui (or Manui) sits to the north of Wawonii. The channel between Menui and Sulawesi is particularly complex geologically, but does not appear to have formed a land bridge during the Pleistocene glaciations (Nugraha and Hall 2018).



## 6.2.2 Sunbirds and the barriers around Wallacea

The sunbirds (Nectariniidae) are a family of small passerines with a distribution extending from Africa in the west to Australia in the east. In a region whose birds have provided the basis for much crucial evolutionary work, sunbirds have often drawn special attention (e.g. Jardine 1843, Wallace 1855, Shelley 1876-1880). Many exhibit strikingly colourful “metallic” plumages (Wallace 1856), which have informed taxonomists of their diversity (Cheke *et al.* 2001). Indeed, the sunbirds as a group “deriv[e] their appellation from their brightly-tinted dress, appearing in higher splendour when played on by the sun-beams” (Jardine 1843). Much remains to be clarified in relation to their evolution however, as species continue to be split on the basis of new sources of information like DNA and bioacoustics (Rheindt 2021). Our understanding of biodiversity continues to develop as we document and identify species-level lineages (Fišer *et al.* 2018).

One challenge to sunbird taxonomy is that many species are widespread and varied in their plumage. The Olive-backed Sunbird *Cinnyris jugularis* (Linnaeus 1766, p.185) is one such member of the family. Under most taxonomic treatments (including Gill *et al.* 2022, followed here), this species ranges from China to northeast Australia, crossing both Wallace’s and Lydekker’s Lines. Gill *et al.* (2022) recognise 21 subspecies across this range. However, it has been suggested that there may be more structure to this population than is currently appreciated, and the Olive-backed Sunbird may warrant treatment as a superspecies. Some recent work (Eaton and Rheindt 2017, Berryman and Eaton 2020b, a, O’Connell *et al.* 2020b, Eaton *et al.* 2021) has introduced the name “Sahul Sunbird *Cinnyris clementiae*” (revived from Lesson 1827) for birds occurring from Sulawesi to the Sahul Shelf. This new species would include, among others, the *plateni* (Blasius 1885) subspecies on Sulawesi and its land-bridge islands, the *frenatus* (Müller 1843) subspecies from Australia and New Guinea, and the *flavigastra* (Gould 1843) subspecies on the Solomon Islands. This is part of a proposed three-way split based on differences in vocalisation and mitochondrial DNA (mtDNA) (Eaton *et al.* 2021), whereby birds on the Sunda Shelf are also elevated to a full species, “Ornate Sunbird *C. ornatus*” (Lesson 1827), and *C. jugularis* is retained for the Philippine population, which includes *obscurior* (Ogilvie-Grant 1894) and *aurora* (Tweeddale 1878) among its subspecies. Lohman *et al.* (2010) had previously proposed that the Philippine population represented a distinct species based on mtDNA, also suggesting the same might be true for Sulawesi populations. Eaton *et al.* (2021) discard the Olive-backed Sunbird common name, referring to the proposed Philippine species as the “Garden Sunbird”. Under the Eaton *et al.* (2021) treatment, Wallace’s Line acts as a boundary between *Cinnyris* sunbird species but Lydekker’s Line does not. However, sampling is incomplete and Eaton *et al.* (2021) highlight that more work is needed to further clarify the taxonomy. Our work aims to contribute to this clarification.

The Black Sunbird *Leptocoma aspasia* (Lesson and Garnot 1828, Dickinson *et al.* 2015) overlaps with the Olive-backed Sunbird in Sulawesi and New Guinea, but it does not occur on the Sunda Shelf and is missing from Australia and the Wakatobi Islands (Figure 6.1). Thus the Black Sunbird's distribution, as currently recognised (BirdLife International 2022), crosses Lydekker's Line but is bounded by Wallace's Line. While there are 21 recognised subspecies of Black Sunbird (Gill *et al.* 2022), including *porphyrolaema* (Wallace 1865) on Sulawesi and its land-bridge islands and the nominate *aspasia* on New Guinea, Eaton *et al.* (2021) do not propose to elevate any of these as species-level splits or "limbo splits".

The divergence of birds on islands is driven by traits of both the islands and the birds. Geographic context may present barriers which will prevent gene flow in some species but not others (Smith *et al.* 2014). Despite the use of the word "lines", biogeographic barriers like Wallace's Line have always been seen as at least partially permeable (Huxley 1868), and the division of biotas by such barriers has been shown to be driven by differences in dispersal ability (White *et al.* 2021). Sunbirds might be expected to be subject to more isolation (and thus evolutionary divergence) compared to other birds: despite their success as island colonists, Wallace (1856) noted that the wings of sunbirds are not suited to long-distance dispersal, being "short and round, quite incapable of any powerful flight". The length and pointedness of a bird's wing is an effective index of its dispersal ability, with important ecological consequences (Sheard *et al.* 2020). Certain birds, known as "great speciators", are adept at colonising islands across relatively short distances, but still diverge readily across their range (Diamond *et al.* 1976). Some sunbirds have displayed a propensity to diverge on the smallest of scales, indeed Philippine sunbirds of the genus *Aethopyga* have diverged both between and within islands (Hosner *et al.* 2013). On a larger scale, Wallace's and Lydekker's Lines have been shown to play a role in the distribution of nectarivorous birds (Carstensen and Olesen 2009).

The Black and Olive-backed Sunbirds of Wallacea make for an interesting biogeographic comparison, as they are two of the only representatives of their family in this region. The sunbirds are believed to have originated in Asia (del Hoyo *et al.* 1992-2013), with only six species occurring in Wallacea (Carstensen and Olesen 2009). The Black and Olive-backed Sunbirds are currently placed in different genera but have been congeneric in the past, and most sunbird genera are thought to be closely related and "not well-marked" in their differences (Cheke *et al.* 2001). The two species share some ecological similarities, including their diet of nectar, fruit, and insects (Cheke *et al.* 2001), but they contrast in ways that are relevant to their dispersal. For

example, the Olive-backed Sunbird occurs more commonly in mangroves than does the Black Sunbird (Eaton *et al.* 2021). Mangrove species are often good island colonisers, either because they are susceptible to being dispersed by the elements or because these marginal habitats are easier to invade (Wilson 1959).

### 6.2.3 Evolution of sunbirds in Southeast Sulawesi

As well as their position between Wallace’s and Lydekker’s Lines, “anomalous” Sulawesi and the Wakatobi Islands are of considerable biogeographic interest in themselves. To date, a lack of genetic data has prevented full assessment of these sunbird populations. Prior to this study, no Black Sunbirds and only three Olive-backed Sunbirds from Sulawesi had been subjected to genetic analysis (Lohman *et al.* 2010). Furthermore, no Olive-backed Sunbird sequences from the Wakatobi Islands have been published before this study. Hartert (1903) named the Wakatobi Sunbird as an endemic species, *Cinnyris infrenata*, noting that it lacked the yellow facial stripes of *Cinnyris jugularis* and had darker plumage on its “upper surface”, particularly the head. This taxon has since been demoted to a subspecies of the Olive-backed Sunbird (Hartert 1920, Gill *et al.* 2022), and its distribution was misrepresented in the literature until recently (Kelly and Marples 2011). Two other Wakatobi endemic species named by Hartert (1903), which were later combined with other species, have recently been recognised as full species once more (Kelly *et al.* 2014, O’Connell *et al.* 2019c). Previous work has noted the morphological and ecological distinctiveness of the Wakatobi *infrenatus* sunbirds, as O’Connell *et al.* (2019a) found they exhibited increased sexual dimorphism and higher population density compared to *plateni* birds from Sulawesi and the land-bridge islands. O’Reilly *et al.* (2018) tested their automatic Bird Vocalisation Difference method on the Olive-backed Sunbird and found diagnosable bioacoustic differences between the Wakatobi and Sulawesi populations. Unpublished genetic work based on the COI and ND3 genes (Kelly 2014) identified the Wakatobi Sunbird as an “incipient species”, which does not share haplotypes with the Sulawesi population, evidence that long-term gene flow has been limited by the barrier surrounding the Wakatobi Islands. However, Kelly (2014) did not find sufficient evidence to advise a species-level split. Eaton *et al.* (2021) highlighted the Wakatobi Sunbird as a “limbo split”, recommending more acoustic and genetic work on them, which this study aimed to provide. Menui’s populations of Black and Olive-backed Sunbirds, on the other hand, were unrecorded in the literature until recently (Monkhouse *et al.* 2018) and so have not been officially assigned to any subspecies. Small, isolated islands like Menui and the Wakatobi hold a disproportionate share of Earth’s biodiversity (Cowie and Holland 2006), so a full understanding of evolution requires sampling of these populations.

## 6.2.4 Aims

On a wide geographic scale, we sought to investigate the effect of Wallace’s and Lydekker’s Lines on sunbirds by studying their population structure across Wallacea and the continental shelves. We hypothesised that this structure would follow one of three general patterns, offering varying levels of support for different taxonomic treatments:

1. A low level of genetic divergence across Wallacea and both continental shelves in the Olive-backed Sunbird, and across Wallacea and the Sahul Shelf in the Black Sunbird, in keeping with the Gill *et al.* (2022) taxonomy. This would suggest that the biogeographic barriers have had only a minor effect on sunbirds, as the Olive-backed Sunbird is subject to neither barrier while the Black Sunbird is subject to only one, Wallace’s Line.

2. Species-level divergence within the Olive-backed Sunbird, with the “Sahul Sunbird” of Wallacea and the Sahul Shelf strongly separated from Sunda Shelf populations, but no strong divergence in the Black Sunbird, supporting the Eaton *et al.* (2021) taxonomy. This would indicate that biogeographic barriers have the same, moderate, impact on both Black and Olive-backed Sunbirds, both species being subject to Wallace’s Line while transcending Lydekker’s Line.

3. Additional diversity and population structure beyond what is indicated by either Gill *et al.* (2022) or Eaton *et al.* (2021). If either the Black or Olive-backed Sunbird exhibits genetic divergence between Wallacea and the Sahul Shelf, for instance, this would indicate a major effect of biogeographic barriers, as both Wallace’s and Lydekker’s Lines have the potential to halt gene flow in sunbirds. If a barrier affects one species but not the other, this raises further questions about how they have evolved and dispersed through the region.

## 6.3 Methods

### 6.3.1 Sampling and DNA sequencing

We carried out mist-netting to sample birds on expeditions between 1999 and 2017 across Southeast Sulawesi, the land-bridge islands off its coast, Menui, and the Wakatobi Islands (Figure 6.1). We followed the methodologies laid out in Redfern and Clark (2001) to measure the following morphological data: wing length (maximum chord), bill length (tip of bill to the base), body mass (in grams), and tarsus length (minimum). We also photographed birds (Figures S6.1 – S6.4) and wrote plumage descriptions in the field. We collected small

numbers of contour feathers from each bird's flank from which to extract DNA. The use of feather samples minimised the risk of injury and avoided disrupting their flight abilities and plumage-based visual signals (McDonald and Griffith 2011). Additional tissue subsamples were provided from the Australian National Wildlife Collection. Feather samples were stored in sealed paper envelopes until DNA was extracted from them. The ANWC tissue subsamples were processed immediately on receipt. All extracted genetic samples were stored at -20°C. In total we produced DNA sequences from 84 Olive-backed Sunbirds: 26 of the *plateni* (Sulawesi) subspecies, 33 of *infrenatus* (Wakatobi), 5 from Menui, and 20 of *frenatus* (15 from Queensland in Australia, 5 from New Guinea). We also produced sequences from 33 Black Sunbirds, including 21 from Sulawesi and its land-bridge islands (*porphyrolaema*), 5 from Menui, and 7 from New Guinea (the nominate *aspasia* subspecies). The New Guinea birds came from three provinces of the nation of Papua New Guinea (PNG): the Western Province on the southern coast of the island, and the Central and Northern Provinces, both located in the southeast of the island. A complete list of samples used in our analyses is available in the Supporting Information (Table S6.1), and all sampling sites are shown in Fig 1.

We used Qiagen DNeasy Blood and Tissue Kits to produce our genetic samples by extracting DNA from feathers and tissue. When working with feather samples we added 5µl of 1M dithiothreitol (DTT) at the digest phase to release the genetic material shielded by keratin in the feather calamus. Otherwise we followed the manufacturer's instructions. Our genetic analyses were based on the mitochondrial genes NADH dehydrogenase subunit 2 (ND2) and NADH dehydrogenase subunit 3 (ND3). Evolutionary histories inferred from mtDNA and nuclear DNA sometimes differ (Rubinoff and Holland 2005, Phillimore *et al.* 2008), but ND2 has shown a particularly high level of agreement with nuclear markers (Campillo *et al.* 2019). Although introgression can obfuscate the patterns explored (Toews and Brelsford 2012), ND2 and ND3 have been found to provide the best phylogenetic resolution in younger radiations (Andersen *et al.* 2015b), and mtDNA's sensitivity to population structure is useful in biogeographic studies (Linck *et al.* 2016). As we aimed to study relatively recent divergences, we deemed ND2 and ND3 appropriate for this investigation. We targeted these two genes using touchdown Polymerase Chain Reactions (PCRs). This technique involved beginning the reaction with an annealing temperature 10°C above the melting temperature of the primers before reducing it gradually, to provide an increased yield compared to standard PCR protocols (Korbie and Mattick 2008). The reagents and quantities we used in our PCRs are shown in Figure S6.5. We amplified the ND3 gene using the L10755-F and DOC-ND3-R1 primer pair (Chesser 1999, O'Connell *et al.* 2019b) and ND2 using internal and external primers of our own design, in order to target the gene in two halves (Figure S5.2, Table S6.2). We screened our PCR products on 2% electrophoresis gels stained with GelRed (Biotium) at a 1X concentration, then had them Sanger sequenced at the GATC Eurofins lab in Köln, Germany. We used the

ClustalW function in BioEdit version 7.2.5 (Hall 1999) to align the resulting sequences and then concatenated ND3 to ND2 in Mesquite version 3.51 (Maddison and Maddison 2018). This gave a total of 1392 base pairs of DNA from each bird.

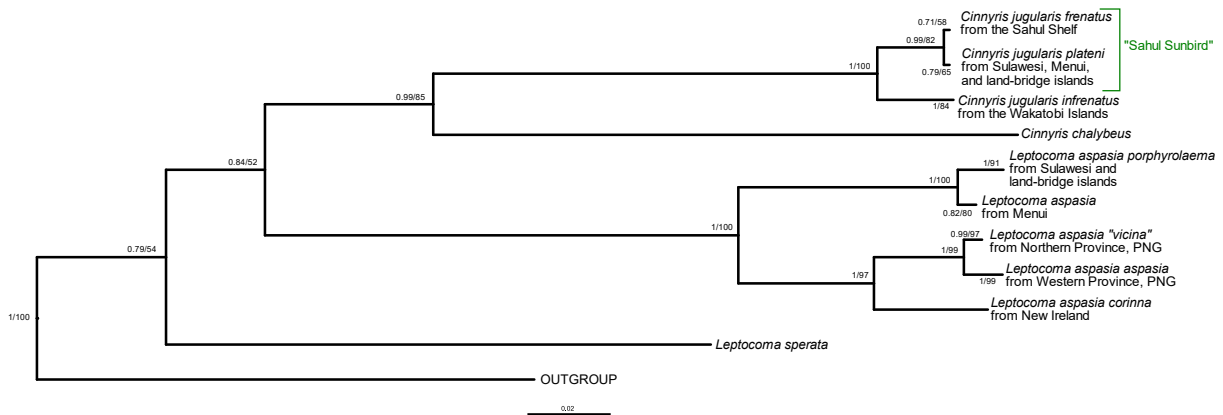
In order to compare genetic distances between our samples and those of other sunbird populations, we downloaded previously published sunbird sequences from GenBank (Table S6.1). We obtained full 1392bp concatenated ND2-ND3 sequences of *Cinnyris chalybeus* from Bowie *et al.* (2016), *Anthreptes malacensis* from Hosner *et al.* (2013), and *Leptocoma sperata* from Moyle *et al.* (2011), and a Black Sunbird sequenced by the B10K Project Consortium (accession number NC\_051024). This bird (USNM-608697) was collected on New Ireland in the Bismarck Archipelago but there has been some confusion as to its subspecific identity, as the museum's online database (Smithsonian 2022) lists it as the *eichhorni* subspecies (Rothschild and Hartert 1926), which is endemic to the Feni islands, while the B10K Project database (B10K Project Consortium 2022) has it as the *corinna* subspecies, which occurs in the rest of the Bismarcks. In addition, we downloaded the 20 Olive-backed Sunbird ND2 sequences that were available. These came from the Sunda Shelf (*ornatus*), the Philippines (*obscurior*, *jugularis*, and *aurora*) and the Solomon Islands (*flavigastra*), allowing us to examine genetic diversity across this range and assess which of our three hypotheses would be supported. These ND2 sequences (from Smith and Filardi 2007, Nyári *et al.* 2009b, Campbell 2013, and Boyce *et al.* 2019) could not be incorporated into our concatenated phylogenies as there was either no corresponding information on ND3 or the ND2 sequences were incomplete (see Table S6.3 for details). Even so, these ND2 sequences allowed us to use a 679bp section of the gene to give an overview of the genetic patterns across a large part of the Olive-backed Sunbird's range.

### 6.3.2 Genetic and phylogenetic analyses

To create a general picture of population structure in Black and Olive-backed Sunbirds, we calculated the pairwise p-distances between ND2 haplotypes, as well as the average genetic distance between populations, using MEGA X version 10.1.8 (Kumar *et al.* 2018) (Tables S6.4 and S6.5). A p-distance or "proportion distance" represents the proportion of varying to matching nucleotide sites across two sequences. These distance measures used the ND2 gene alone, to make them more widely comparable. We investigated the phylogenetics of sunbirds using both Maximum Likelihood (ML) and Bayesian approaches. We applied these methods, separately, to our concatenated 1392bp ND2-ND3 haplotypes and to the shorter 697bp alignment that included birds from a larger part of the Olive-backed Sunbird range. Phylogenies included one

representative of each concatenated ND2/ND3 haplotype. We used the ModelFinder function (Kalyaanamoorthy *et al.* 2017) in IQTree version 1.6.12 (Nguyen *et al.* 2015) to select the most appropriate nucleotide substitution model for our alignment. Choosing an appropriate nucleotide substitution model, which defines the probability of a given nucleotide base in the gene changing to another, is important in calculating the likelihood of a phylogenetic tree (Sullivan and Joyce 2005).

We carried out ML analysis on our concatenated ND2/ND3 sequences in IQTree version 1.6.12 (Nguyen *et al.* 2015) using the GTR+F+I+G4 model. The group support of the ML phylogeny was based on 1000 bootstraps, resampling points from our dataset and counting how many of these bootstrap trees supported each monophyletic grouping (Felsenstein 1985). We used MrBayes version 3.2.7 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) to carry out Bayesian phylogenetic inference on our concatenated ND2-ND3 sequences, a technique to construct phylogenies using the Markov Chain Monte Carlo (MCMC) method (Mau *et al.* 1999). We selected a GTR+G+I model, the closest equivalent to our ML model, with two MCMC runs and four chains per run, sampling every 1000 generations. As the MCMC algorithm converges on the data over time, with the time it spends on a given tree equivalent to the Bayesian posterior probability, i.e. the probability that said tree is correct (Huelsenbeck *et al.* 2001), we discarded the first 25% of each MCMC run as a “burn-in” (Mau *et al.* 1999). It is also important to confirm that the MCMC algorithm has “converged” on a good approximation of the data and not just a local maximum (Besag and Green 1993). We assessed convergence by reading the Bayesian model’s output parameters in TRACER version 1.7.1 (Rambaut *et al.* 2018). Following O’Connell *et al.* (2019c), we deemed it acceptable once average standard deviation in split frequencies (ASDSF) reached 0.01 and Effective Sample Size (ESS) of model parameters reached 200. Both requirements had been surpassed by the time our MCMC algorithm had iterated 4 million generations. We exported a 50% majority rule consensus tree from MrBayes (Figures 6.3c and 6.4c) and imported both this and the ML tree into the R package “ggtree” (Yu *et al.* 2016), which we used to combine both trees into a single figure (Figure 6.2). The appendices include full, separate Bayesian (Figure S6.6) and ML (Figure S6.7) trees, with all outgroup and ingroup haplotypes represented.



**Figure 6.2.** Simplified version of a combined Maximum Likelihood (ML) and Bayesian phylogenetic tree of *Cinnyris* and *Leptocoma* species sampled in Wallacea and the Sahul Shelf. In this figure the outgroup is omitted and each major clade in the data is collapsed into a single branch. Nodes are labelled with ML bootstraps / Bayesian probability.

Full versions of the ML and Bayesian trees, including all outgroup taxa, are provided in the Supplementary Material (Figures S6.6 and S6.7).

We created Templeton-Crandall-Sing (TCS) haplotype networks (Figures 6.3b and 6.4b) using PopArt version 1.7 (Leigh and Bryant 2015) to visualise the genetic structure of our sunbird populations. The TCS method uses an agglomerative algorithm, working progressively to combine clusters sharing one or more connecting edge (Templeton *et al.* 1992). This makes it ideal for the analysis of divergence at the population level, as ancestral haplotypes are likely to be common in the population and variation tends to be lower compared to analyses at higher taxonomic levels (Clement *et al.* 2000).

### 6.3.3 Molecular species delimitation

We used Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.* 2012) to perform distance-based molecular species delimitation on our sunbird sequences. This method divides sequences into putative “species” with smaller genetic distances within than between them. ABGD calculates a minimum threshold interspecific distance, the “barcode gap”, which can be calibrated based on the expected prior intraspecific divergence of the gene or taxon. We ran our ABGD analysis on the web-server (<https://bioinfo.mnhn.fr/abi/public/abgd/>) using the default Kimura-2-Parameter (K2P) model and settings (Pmin = 0.001, Pmax = 0.1, Steps = 10, relative gap width = 1.5, Number of bins = 20). Our main ABGD analysis included all our focal *Cinnyris jugularis* and *Leptocoma aspasia* samples, as well as the congeneric *Cinnyris*



*chalybeus* and *Leptocoma sperata* sequences downloaded from GenBank. As ABGD is a distance-based method, we restricted it to a single gene, ND2. We also ran a second ABGD analysis using the partial Olive-backed Sunbird ND2 sequences from GenBank, in a 679bp alignment with our focal Olive-backed Sunbird sequences, to assess population structure across this wider range and see if it would support species status for the Philippine and Sunda Shelf populations (Eaton *et al.* 2021).

### 6.3.4 Bioacoustic and morphological analyses

Bioacoustics are an increasingly important aspect of avian species delimitation, and this approach has previously been recommended to help clarify the status of the Wakatobi Olive-backed Sunbird population (Eaton *et al.* 2021). We recorded 68 Olive-backed Sunbirds on Sulawesi and the Wakatobi Islands using a Zoom H2 Handy Recorder, with a Sennheiser Me62 external microphone and a Telinga V2 parabolic reflector. The analysis was carried out on calls rather than the more complex mating songs, as we had more recordings of these. Songs are commonly used in assessing pre-mating reproductive isolation, but a difference in calls might be stronger evidence of speciation, as calls tend to change more slowly (Marler 2004). Changes in calls are sometimes driven by changes in body mass, however (Potvin 2013). We used Raven Pro version 1.6 (Center for Conservation Bioacoustics 2019) to prepare spectrograms from these calls and extracted data on standard acoustic traits (Tobias *et al.* 2010). We used both temporal features; namely duration, number of notes, and pace (number of notes divided by duration), and spectral features; specifically maximum frequency, minimum frequency, bandwidth (maximum frequency minus minimum frequency), and peak frequency (frequency with highest amplitude). To account for the variation in vocalisations within individual birds, individual means were calculated for the calls in each recording. The mean data for each recording were then used as sample points. We analysed these acoustic data using R version 4.1.1 (R Core Team 2021) using the methods described below.

Only adult birds processed by the same author were included in morphological analyses, and males and females were treated separately due to sexual dimorphism. This provided a morphological sample size of 147 male Olive-backed Sunbirds (41 from Sulawesi and its land-bridge islands, 106 from the Wakatobi Islands) and 95 female Olive-backed Sunbirds (25 from Sulawesi and its land-bridge islands, 69 from the Wakatobi Islands, 1 from Menui). The one Olive-backed Sunbird from Menui was grouped with the Sulawesi birds based on genetic results. Our morphological analysis on the Black Sunbird used female birds as the

sample was larger (27 from Sulawesi and its land-bridge islands, 7 from Menui). We used Multivariate Analysis of Variance (MANOVA) on our morphological and bioacoustic data to investigate whether these would support the clades suggested by our genetic work. We used histograms and Pearson's  $r$  to check that the assumptions of MANOVA were not violated, i.e. that the residuals were normal and that the dependent variables were linearly related without being overly strongly correlated ( $0.1 < r < 0.8$ ). We generated boxplots (Figures S6.8 – S6.11) to visualise morphological and acoustic differences between our populations of interest.

We also subjected our bioacoustic and morphological data to a points-based integrative species delimitation system (Tobias *et al.* 2010). This system has been widely used by organisations such as BirdLife International, the global authority for birds for the IUCN Red List, and reference works such as the Handbook of the Birds of the World (del Hoyo *et al.* 1992-2013). It has been criticised by some authors (Rheindt and Ng 2021), however, and is best combined with multiple lines of evidence, including genetic data (Tobias *et al.* 2021). The system allows a difference between populations to be scored on morphology, acoustics, plumage, and some other sources of information that weren't applicable to our study. A score of seven (or more) points indicates a species-level difference between the populations being compared. The Tobias *et al.* (2010) system uses effect sizes (Cohen's  $d$ ) to build "score" values. Effect sizes are considered to provide a more objective measure than p-values for statistical assessments in biology (Nakagawa and Cuthill 2007). We used the R package "effsize" (Torchiano 2020) to calculate Cohen's  $d$  for the morphological and acoustic differences between the Sulawesi (including the land-bridge islands) and Wakatobi populations of the Olive-backed Sunbird, and scored the plumage differences using our photographs and field descriptions. We used male plumages for the taxonomic scoring as this forms the basis of the current taxonomy of subspecies in the Olive-backed Sunbird. Our field descriptions covered 10 males from Sulawesi and the land-bridge islands and 31 males from the Wakatobi Islands. We also examined photographs of each of these birds.

Finally, we calculated the dispersal index of our Olive-backed Sunbirds following Ó Marcaigh *et al.* (2022b) and O'Connell *et al.* (2019c), using the wing length and weight data. We ran a Welch's two-sample t-test to see if sunbirds on the Wakatobi Islands differed in dispersiveness from their relatives on the land-bridge islands, as loss of dispersiveness has been described in other birds on similarly isolated islands (Pedersen *et al.* 2018, Pepke *et al.* 2019). More information on this index is provided in Appendix S6.1, and it is plotted in Figure S6.11.

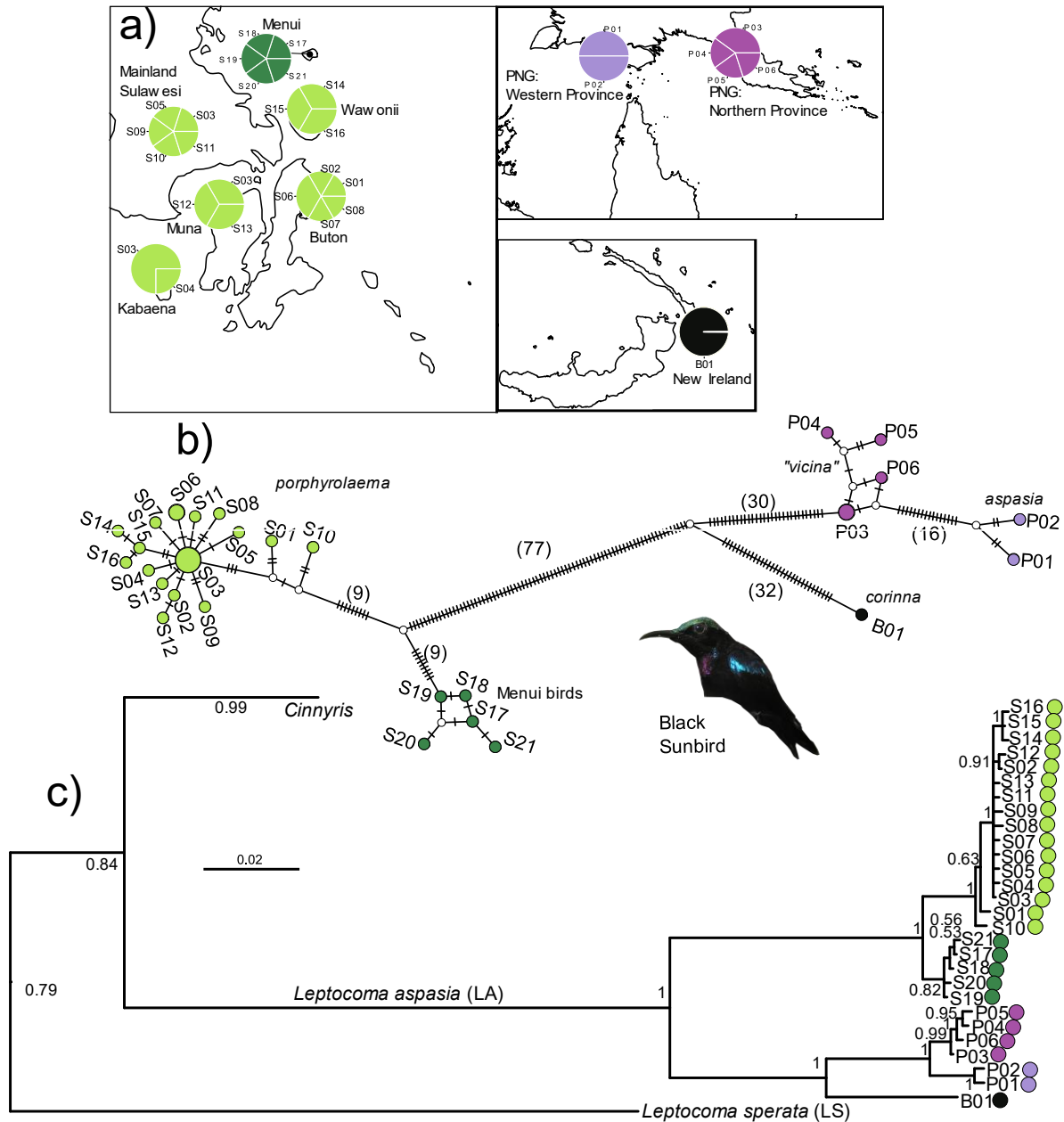
## 6.4 Results

### 6.4.1 Genetic and phylogenetic results

Our ModelFinder analysis found strong support for a General Time Reversible (GTR) model with a gamma shape parameter and a proportion of invariable sites (GTR+F+I+G4), indicating that mutations in our selected genes can occur but subsequently reverse as time passes (Lanave *et al.* 1984, Tavaré 1986). Biological constraints prevent change in certain parts of the gene, termed invariant sites (Waddell and Steel 1997). Our model selection was supported by lower Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) than other models. A lower AIC indicates that less information is being “lost” by the model, while a lower BIC indicates a “better fit” (Johnson and Omland 2004).

Our haplotype networks and phylogenetic trees revealed substantial geographic population structure in both the Olive-backed and Black Sunbirds. In the Olive-backed Sunbird (Figures 6.2 and 6.3), the *plateni* birds from Sulawesi and its land-bridge islands were seen to be closely related to the *frenatus* birds from Australia and PNG. The *infrenatus* birds from the Wakatobi Islands formed their own cluster. In the Black Sunbird network and tree (Figures 6. 2 and 6.4), the Black Sunbirds from PNG formed a clade, while a second clade included the birds from Sulawesi, Menui, and the land-bridge islands. The single *corinna* bird from New Ireland formed a sister clade to the PNG birds. Our tree and network showed separate Western Province (LA\_P01 and P02) and Northern Province (LA\_P03-06) clusters in New Guinea in the Black Sunbird. The Black Sunbirds from Menui were separated from the rest of the Sulawesi cluster by a similar number of mutations as the populations in mainland PNG were separated from each other.





**Figure 6.4.**

**a)** Geographic distribution of *Leptocoma aspasia* haplotypes in Wallacea and the Sahul Shelf. Each circle represents an island and the fractions within the circle the haplotypes found on that island, proportioned to represent the frequency of each haplotype. The haplotypes are named according to the species-level divisions suggested by ABGD and coloured to represent the clades supported by our phylogenetic analyses.

**b)** TCS Haplotype Network of *Leptocoma* haplotypes. Each circle represents a unique ND2-ND3 haplotype, sized to represent how many birds carried that haplotype. The hatch marks represent mutations between haplotypes, also given as numbers in brackets for the wider divergences. The unfilled, white nodes represent hypothetical ancestral states.

**c)** Bayesian consensus tree of *Leptocoma* haplotypes. Nodes are labelled with Bayesian probabilities.

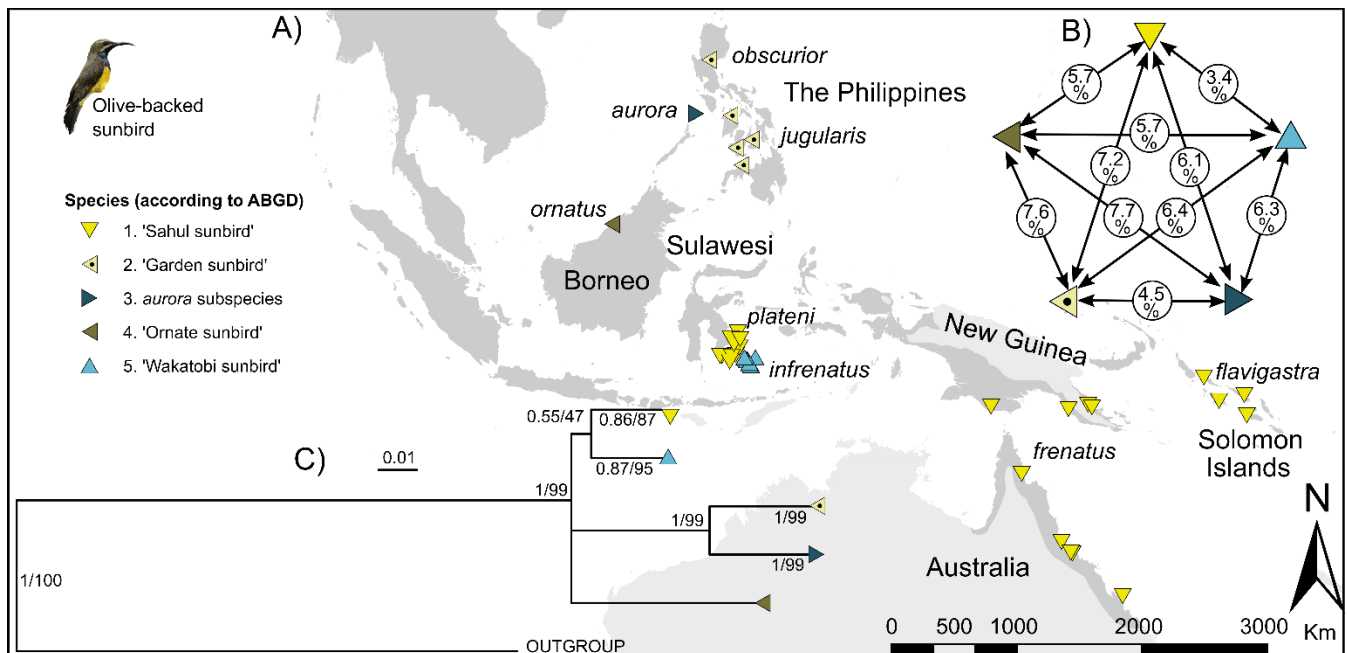
ABGD found strong support for species-level splits within both Black and Olive-backed Sunbirds. The most favoured partitioning of the ND2 sequences, compatible with the widest range of prior intraspecific divergences, produced seven groups with a barcode gap of 2.2%. The first group, CJ\_S, contained all Olive-backed Sunbirds from Sulawesi, its land-bridge islands, Menui, PNG, and Australia. The second, CJ\_W, contained all Olive-backed Sunbirds from the Wakatobi Islands. The *Cinnyris chalybeus* outgroup sequence was grouped by itself. The fourth group, LA\_S, contained all Black Sunbirds from Sulawesi, its land-bridge islands, and Menui. The fifth group, LA\_P, contained all Black Sunbirds from the mainland provinces of PNG. The single Black Sunbird sequence from the Bismarcks was grouped by itself as LA\_B. The seventh “group” contained the sole sequence of *Leptocoma sperata*.

We found a mean genetic distance of 9.1% between the LA\_S group of Black Sunbirds and the LA\_P group. Within these populations, there was a mean genetic distance of 1.4% between Menui and Sulawesi (including the land-bridge islands), and a mean distance of 1.4% between the Northern and Western Provinces of PNG. The bird from the Bismarcks (LA\_B) had a mean genetic distance of 9.2% from the LA\_S birds and 4.9% from LA\_P. Mean intrapopulation genetic distance was equal to 0.1% in the Menui population, 0.2% in Sulawesi and the land-bridge islands, 0.1% in the Northern Province of PNG and 0.3% in the Western Province of PNG.

Within Wallacea, the mean genetic distance ( $p$ -distance) between Olive-backed Sunbirds caught on the Wakatobi Islands and those from Sulawesi, Menui, and the land-bridge islands was 3.3%. The mean genetic distance between the Wakatobi and Sahul Shelf populations was 3.1%. Meanwhile, the mean distance between the Sulawesi and Sahul Shelf populations was just 0.6%, leading us to group these together as the “Sahul Sunbird” in further analyses. The mean intra-group genetic distances were 0.2% for the Wakatobi, 0.4% for Sulawesi and the land-bridge islands, and 0.2% for the Sahul Shelf.

Our analyses using a portion of the ND2 gene to assess a wider part of the Olive-backed Sunbird range also found strong evidence of species-level splits (Figure 6.5). Using 697bp of ND2, ABGD found a strong consensus for five species in the Olive-backed Sunbird superspecies, with a barcode gap of 2%. The first group contained all of the “Sahul Sunbird” (Eaton *et al.* 2021) sequences, including our *platani* samples from Sulawesi, its land-bridge islands, and Menui, our *frenatus* samples from PNG and Australia, and the partial sequences of *flavigastra* published by Smith and Filardi (2007) from the Solomon Islands. The second

group (“Garden Sunbird”) contained all sequences of the *jugularis* and *obscurior* subspecies from the Philippines. The two birds of the *aurora* subspecies from Busuanga in the western Philippines formed a third group. The single *ornatus* sequence from Borneo was grouped by itself (“Ornate Sunbird”). All of our *infrenatus* sequences from the Wakatobi Islands made up the fifth and last group (“Wakatobi Sunbird”). Each of the “species” assigned by ABGD based on 697bp of ND2 formed its own well-supported clade in the partial ND2 Bayesian and ML trees (Figure 6.5c). In this 697bp portion, there was a mean genetic distance of 4.5% between the *aurora* subspecies and the “Garden Sunbird”. The “Garden Sunbird” exhibited a mean genetic distance of 6.4% from the “Wakatobi Sunbird”, a distance of 7.6% from the “Ornate Sunbird” sequence and a 7.2% mean distance from the “Sahul Sunbird”. The *aurora* subspecies had a mean genetic distance of 6.3% from the “Wakatobi Sunbird” and a 6.1% mean distance from the “Sahul Sunbird”. The “Ornate Sunbird” sequence exhibited a 7.7% mean genetic distance from the *aurora* sequences, a distance of 5.7% from the “Wakatobi Sunbird”, and a 5.7% distance from the “Sahul Sunbird”.



**Figure 6.5.**

**a)** Map of the Indo-Pacific with shading representing the range of the Olive-backed Sunbird shaded, as currently recognised by BirdLife International. Sampling sites of the birds included in our 679bp partial ND2 analysis are marked with different triangles, according to the species they were assigned to by ABGD. Currently recognised subspecies are labelled (Gill *et al.* 2022).

**b)** Mean genetic distance (uncorrected p-distance) between each of the species recognised by ABGD, based on a 679bp partial ND2 alignment.

#### 6.4.4 Results of bioacoustic and morphological analysis

We carried out an integrative taxonomic analysis Tobias *et al.* (2010) of the “Wakatobi Sunbird” by comparing the plumage, morphology, and acoustics of this population to those of the neighbouring “Sahul Sunbird” populations of Sulawesi, its land-bridge islands, and Menui. The Tobias *et al.* (2010) system allows up to three plumage characters to be scored. Eaton *et al.* (2021) highlight two plumage differences between the Wakatobi Islands and the “Sahul Sunbird”: they describe the back of Wakatobi birds as “brown” rather than olive and they note that their face is plain where the “Sahul Sunbird” has distinct yellow moustachial stripes and supercilium. Our photographs (Figures S6.1 and S6.2) and field descriptions found back colour to be somewhat variable in both populations, but the Wakatobi birds were generally darker. Under the Tobias *et al.* (2010) system we rank this as a minor difference (“a slightly different wash or suffusion to all or part of any area”). We found that the faces of males from the Wakatobi Islands are distinctly darker, with the lack of markings presenting a marked contrast. We categorised this as a major difference (“contrastingly different hue/colour to all or part of a significant area”). Together, these plumage characters are worth four points.

The Tobias *et al.* (2010) system allows the scoring of two morphological characters, the largest increase and the largest decrease. The largest morphological decrease in the male Olive-backed Sunbird population of the Wakatobi Islands was in bill length (Cohen’s  $d$  of -0.9), which is classed as a “minor” difference and provides a score of one point each towards the total. The largest increase in the male Wakatobi birds was in tarsus length (Cohen’s  $d$  of 2), which is classed as a medium difference, worth two points towards the total. These traits are plotted in Figure S6.8.

This system also allows the scoring of two acoustic characters, one spectral and the other temporal. We found the largest spectral effect size was in bandwidth (Cohen’s  $d$  of 1.4, with the “Sahul Sunbirds” singing with larger bandwidths) and the largest temporal effect size was in pace (Cohen’s  $d$  of 0.5, with the “Sahul Sunbird” singing at faster paces). These are both considered “minor” differences, worth one point each toward the total. These traits are plotted in Figure S6.10.

This gives a total “Tobias score” across all characters of nine, exceeding the threshold of seven points, indicating a species-level difference between the Wakatobi birds and the “Sahul Sunbird”. Using female birds instead of males for the morphological analysis would have given a total of eight, still surpassing the threshold.



The difference between the “Wakatobi Sunbird” and the “Sahul Sunbird” (specifically the Olive-backed Sunbird populations of Southeast Sulawesi and its land-bridge islands) was reinforced by the bioacoustic and morphological MANOVA results. Only peak frequency, minimum frequency, and bandwidth could be included in the bioacoustic MANOVA, as the other variables were either not linearly related or too strongly correlated (Pearson’s  $r > 0.8$  or  $< 0.1$ ). This MANOVA revealed that the “Wakatobi Sunbird” is strongly differentiated in song from the “Sahul Sunbird” ( $p < 0.001$ , full output in Table S6.6). The morphological MANOVA included the wing length, bill length, weight, and tarsus length data, and found that the “Wakatobi Sunbird” is strongly differentiated from the “Sahul Sunbird” in these traits ( $p < 0.001$ , full output in Tables S6.8 and S6.10). This result was consistent, no matter whether male or female birds were used.

Our Welch’s t-test found that the “Wakatobi Sunbird” had a considerably lower dispersal index than the “Sahul Sunbird” populations of Southeast Sulawesi, its land-bridge islands, and Menui ( $p < 0.00005$  in males,  $p < 0.005$  in females).

As the Menui Black Sunbird population was suggested as a subspecies by our genetic work, we used MANOVA to assess whether this population was distinct from Sulawesi and the land-bridge islands in wing length, bill length, weight, and tarsus length (all of these variables being acceptably correlated). This analysis used female birds as only two adult male Black Sunbirds were caught on Menui (versus seven adult females). We found that female Black Sunbirds are morphologically distinct on Menui compared to the land-bridge islands ( $p < 0.001$ , full MANOVA outputs in Table S6.9, plotted in Figure S6.9). We did not carry out Tobias scoring on the Black Sunbird as there were no clear plumage differences and fewer song data were available.

## 6.5 Discussion

### 6.5.1 *Cinnyris* taxonomy

Our results indicate that the Olive-backed Sunbird represents a superspecies and should be split into at least four species. We have found support for the three-way split suggested by Eaton *et al.* (2021) while also

providing the first genetic, acoustic, and morphological evidence that the “Wakatobi Sunbird” warrants recognition as an additional species. The Wakatobi population exhibits a strikingly non-linear arrangement of population structure, with a range enveloped within that of the more widespread species, and had previously been suggested as a “limbo split” (Rheindt 2021) based on plumage differences alone (Eaton *et al.* 2021). Our study has supported these differences with matching patterns in mtDNA and integrative species delimitation or “Tobias scoring” (Tobias *et al.* 2010). Due to the lower effective population size of mtDNA, along with other factors, differences in mtDNA should be integrated with other forms of evidence in this way (Rubinoff and Holland 2005). In the light of this new integrative evidence, we recommend that the “Wakatobi Sunbird *Cinnyris infrenatus*”, originally named by Hartert (1903), be reinstated as a separate species. In addition to its genetic divergence (Figure 6.3), the “Wakatobi Sunbird” has shorter wings, a shorter bill, and longer tarsi than the “Sahul Sunbird” (Figure S6.8), as well as exhibiting slower and higher pitched calls over a smaller bandwidth (Figure S6.10). This study is one of several to have remarked upon the distinctiveness of the Wakatobi avifauna (Kelly *et al.* 2014, O’Connell *et al.* 2019a, O’Connell *et al.* 2019c), and so we reiterate the recommendation of O’Connell *et al.* (2020b) that the Wakatobi Islands should be protected as an Endemic Bird Area (Stattersfield *et al.* 1998).

In addition to the distinctive nature of the Wakatobi lineage, our work supports the splits suggested by Eaton *et al.* (2021) and one possible additional split in the Philippines (Figure 6.5). Under the Eaton *et al.* (2021) treatment, populations from Sulawesi to the Sahul Shelf and the Solomon Islands are named as a new species, the “Sahul Sunbird *Cinnyris clementiae*”, the Sunda Shelf populations become “Ornate Sunbird *Cinnyris ornatus*”, and the Philippine birds retain the *Cinnyris jugularis* name and take “Garden Sunbird” as a common name. This is supported by deep genetic divergences (all greater than 5%) between these three putative species, with the Sahul Shelf represented by our new Sulawesi, Australian, and PNG sequences along with Solomon Islands birds from Smith and Filardi (2007), the Sunda Shelf by a sequence from Borneo (Boyce *et al.* 2019), and the Philippines by a number of previously published partial sequences (Table S6.3). While this split has been suggested previously (Eaton *et al.* 2021) based on limited sampling and incomplete sequences, our more comprehensive sampling of full sequences, taken from both ends of the new “Sahul Sunbird” species range, offers stronger support for the division. Our study has also suggested another potential split in this species complex (Figure 6.5), outside the geographic range covered in detail by Eaton *et al.* (2021). ABGD considered the *aurora* sequences from Busuanga in the western Philippines to represent a distinct species, with a mean genetic distance of 4.5% from the greater Philippine archipelago (subspecies *obscurior* and *jugularis*). Ornithologists (Rand 1951, Billerman *et al.* 2022) have grouped the *aurora* subspecies separately from these other Philippine subspecies due to its orange breast plumage. The western

chain of islands on which the *aurora* subspecies occurs is geologically and biogeographically distinct from the greater Philippine archipelago (Diamond and Gilpin 1983). The *aurora* subspecies displayed a similar level of genetic divergence to that of the more thoroughly sampled “Wakatobi Sunbird”, but was represented in our analysis by two partial ND2 sequences from Campbell (2013), and so we recommend further sampling of this population.

The lack of divergence between the Menui population and the wider Southeast Sulawesi population in the “Sahul Sunbird” confirms that the Menui population belongs to the *plateni* subspecies. On the Sahul Shelf itself, our “Sahul Sunbirds” exhibit a uniform population across PNG and Australia. This is in keeping with the current assignment of these populations to one subspecies, *C. j. frenatus*.

### 6.5.2 *Leptocoma* taxonomy

The movement of species between the genera *Leptocoma*, *Cinnyris*, and *Nectarinia* has previously caused confusion for taxonomists working with the Black Sunbird (LeCroy 2010). Our analyses suggest that the *Leptocoma* genus may not be monophyletic (Figure 6.2). However, our trees’ genus-level branches had lower support than the species- and subspecies-level branches: as discussed above, mtDNA is more suitable for analysing recent divergence. We strongly recommend further sequencing of all species within *Leptocoma*, to clarify the genus taxonomy.

Previous research has not proposed any splits or “limbo splits” within the Black Sunbird (Eaton *et al.* 2021). Nonetheless, we found that the Black Sunbird exhibited genetic divergence consistent with a species-level split between Wallacea and the Sahul Shelf, with a high genetic distance between Sulawesi and PNG (9.1%). This presents a marked contrast to the “Sahul Sunbird”, which exhibited minimal divergence between those same populations. Further sequencing of birds from intervening areas (such as the Maluku Islands) would help to clarify the geographical boundaries between these potential species. As several intervening populations are absent from our analysis, we cannot rule out the possibility of clinal variation between the Sulawesi and PNG populations (Brumfield 2005, Cros and Rheindt 2017). However, the genetic divergence we found was so strong (mean p-distance of 9.1%) that it seems more likely the two populations represent two species. The patterns we have observed suggest a division along Lydekker’s Line, and so we predict that sampling of the intervening populations would show a Wallacean species occurring from Sulawesi to the Maluku Islands and a separate species on New Guinea. This would involve the elevation of one of the

Wallacean subspecies names to species level, as Lesson and Garnot (1828) named the Black Sunbird initially for a specimen from Manokwari (Doréry) on the New Guinea mainland. The discovery of cryptic species within the Black Sunbird highlights the importance of comprehensive sampling of species across their range, even in the absence of obvious plumage differences.

The structure within the Black Sunbird contrasted with the patterns shown by the Olive-backed Sunbird and “Sahul Sunbird” in several ways. The Black Sunbird exhibited a split between Sulawesi and PNG, where the “Sahul Sunbird” had a continuous population. The Black Sunbird also exhibited structure within both of these regions. Most taxonomic treatments (Cheke *et al.* 2001, Billerman *et al.* 2022) split the Black Sunbird into distinct subspecies in PNG’s Western Province (*L. a. aspasia*), Northern Province (*L. a. vicina*), and the Bismarcks (*L. a. corinna*). However, Gill *et al.* (2022) merged *L. a. vicina* into *L. a. aspasia*, following Rand (1967). Our genetic work supports *L. a. aspasia* and *L. a. vicina* as distinct subspecies, while *L. a. corinna* appears to warrant species status based on ABGD. However, as this was based on a single sequence we recommend that the Bismarck populations be examined further before a judgement is made. The Menui Black Sunbird population was genetically distinct from Sulawesi and the land-bridge islands, with a mean genetic distance (1.72%), a distance greater than that between the two subspecies in PNG (1.48%). This population was also found to be distinct in wing length, bill length, weight, and tarsus length (MANOVA,  $p < 0.001$  in females). These findings suggest the Menui population of Black Sunbird may warrant recognition as an endemic subspecies.

### 6.5.3 Implications for biogeography and evolution

The division between the “Sahul Sunbird” in Sulawesi and the “Ornate Sunbird” in neighbouring Borneo (Eaton *et al.* 2021) reflects one of the most iconic patterns in biogeography. Between these islands runs the boundary between the shallow Sunda Shelf and the deeper waters of Wallacea, which corresponds to the original and best-known version of “Wallace’s Line” (Wallace 1863, Figure 6.1). However, Wallace’s Line, Wallacea, and related biogeographic ideas have been treated in a “tangled” way in the literature, the treatment of Sulawesi and the Philippines being particularly contentious (Ali and Heaney 2021). The three-way split of the Olive-backed Sunbird (Eaton *et al.* 2021), which our work supports, reinforces the more widely used versions of Wallace’s Line (west of Sulawesi, southeast of the Philippines) and Wallacea

(excluding the Philippines), as there are separate species in Wallacea (“Sahul Sunbird”), the Philippines (“Garden Sunbird”), and the Sunda Shelf (“Ornate Sunbird”).

Unlike Wallace’s Line, a similar barrier in the east has not prevented gene flow in this species, as the “Sahul Sunbird” appears to cross Lydekker’s Line with only shallow divergence between populations on either side. It is remarkable that the “Sahul Sunbird” appears to maintain a range all the way from Sulawesi to Australia, while the “Wakatobi Sunbird” seems to have diverged in a small archipelago nested within this range. Buton is only 27km from the Wakatobi Islands, while the stretch of islands between Sulawesi and New Guinea contains deep-water barriers up to 100km wide (this largest barrier is that between Misool and Seram). This striking pattern appears to conform with the “Taxon Cycle” (Wilson 1961, Ricklefs and Bermingham 2002), whereby taxa become less dispersive as they age. In this framework, the “Sahul Sunbird” would occupy an early stage of the Taxon Cycle, leading to a dispersive lifestyle and a relatively recent colonisation of the Sahul Shelf (or perhaps a back-colonisation of Sulawesi) across Lydekker’s Line. This would be in keeping with previous hypotheses that the Olive-backed Sunbird colonised the Sahul Shelf from Wallacea quite recently (Mayr 1942, Schodde 1977). The “Wakatobi Sunbird” would occupy a later stage of the Taxon Cycle, maintaining a sedentary lifestyle in a small range. The “Wakatobi Sunbird” may thus represent a biological species, as it would have maintained its genetic and phenotypic divergence during this recent colonisation wave. In other bird families that are widespread in this region, populations on small oceanic islands have been subject to selection for reduced dispersal ability, leading to a pattern of widespread continental species and isolated island relatives (Pedersen *et al.* 2018, Pepke *et al.* 2019). We have found evidence that this might also be the case in sunbirds, as the “Wakatobi Sunbird” exhibited a significantly lower dispersal index than the “Sahul Sunbird” populations of Sulawesi, its land-bridge islands and Menui. Since the earliest days of biogeography (Wallace 1880), small, isolated “oceanic” islands like the Wakatobi Islands have been known to harbour disproportionate numbers of endemic species. Birds on smaller islands are subject to different evolutionary processes than those on continents, exhibiting lower effective population sizes and losing fewer deleterious mutations to natural selection (Leroy *et al.* 2021).

This study also demonstrates the somewhat stochastic nature of island colonisation, as we have found that the sunbirds of Runduma colonised this island (located over 50km east of the main Wakatobi archipelago) from an entirely different direction than the island’s white-eyes. These are among the only two small passerines on Runduma that aren’t island specialists or human commensals. The “Wakatobi Sunbird” arrived on this tiny island from the Wakatobi Islands: CJ\_W03 was the only haplotype found on Runduma,

and is shared with Hoga, Tomia, Binongko, and Lintea (Figure 6.3). Runduma's white-eye population, on the other hand, is most closely related to that of mainland Southeast Sulawesi (O'Connell *et al.* 2019c). Despite their evolutionary importance, many small islands in the Indo-Pacific have received little ornithological attention until recently (Monkhouse *et al.* 2018, O'Connell *et al.* 2020b, Sin *et al.* 2022).

The Black Sunbird was already known to be subject to Wallace's Line, as Sulawesi represents the western extent of its range. Cheke *et al.* (2001) hypothesised that the Black Sunbird might be part of a superspecies, with its sister species on the other side of Wallace's Line. Unlike the "Sahul Sunbird", our work indicates that *Leptocoma* sunbirds have speciated over Lydekker's Line, based on the strength of divergence between populations on Sulawesi and PNG. Where the "Sahul Sunbird" exhibits a similar population throughout Australia and PNG, the Black Sunbird exhibits noticeable structure on this scale. That the Bismarcks hold an endemic taxon illustrates once again the importance of small and isolated islands in generating biodiversity through speciation.

#### 6.5.4 Conclusion

We have found additional diversity and population structure in sunbirds beyond what is indicated by either Gill *et al.* (2022) or Eaton *et al.* (2021), i.e. the third of our hypothesised general patterns. Our findings support Eaton *et al.* (2021) in splitting the Olive-backed Sunbird, which is treated as one species by Gill *et al.* (2022), but where Eaton *et al.* (2021) split the species in three, our work supports the "Wakatobi Sunbird" as a fourth species. We have also identified cryptic populations in the Black Sunbird, which were not suggested by either of these taxonomic treatments, with a stronger mitochondrial divergence between Wallacean and Sahul Shelf *Leptocoma* populations than between *Cinnyris* species. That Lydekker's Line acts as a biogeographic barrier to the Black Sunbird but not the "Sahul Sunbird" is curious, perhaps reflecting a recent colonisation or back-colonisation across the Line by the "Sahul Sunbird". The Olive-backed Sunbird may be a stronger disperser than the Black Sunbird overall, as its tendency to occur in mangroves (Eaton *et al.* 2021) would indicate. The Cicadabird *Edolisoma tenuirostre* undertook a similarly recent colonisation of this region, resulting in the entire Sahul Shelf being inhabited by an Australo-Papuan clade with strong dispersal abilities (Pedersen *et al.* 2018). Perhaps, over time, species-level divergence could occur between the "Sahul Sunbird" populations separated by Lydekker's Line.

Sunbirds have been admired by naturalists for centuries, and by artists for even longer: Olive-backed Sunbirds are depicted in reliefs on Java's Borobudur Temple, dating to the 8<sup>th</sup> or 9<sup>th</sup> century CE, their search for nectar representing the Buddhist quest for enlightenment (Ashari *et al.* 2021). However, improved sampling and modern integrative methods are only now revealing the true diversity within these species. As more island populations are sampled, such as those on the Wakatobi Islands, Menui, and the Bismarcks, we learn more of their important role in evolutionary processes. It is interesting to contrast these effects with the large-scale barriers like Wallace's and Lydekker's Lines. From tiny archipelagos to massive continental shelves, the threatened biodiversity of Wallacea and the Indo-Pacific needs to be examined at multiple scales if we are to be enlightened to its evolutionary history (Cicero *et al.* 2021) and conservation (Myers *et al.* 2000).

## Chapter 7 - The genetic divergence between bird populations on islands is driven by both ecology and geology

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*"The water is wide, I cannot cross o'er  
And neither have I the wings to fly..."*  
- Traditional Scottish/Irish folk song

**Author Contribution:** I have led the work that forms the basis of this chapter, but it has been carried out as a collaborative effort. The initial idea was conceived from conversations with Albert Phillimore. With input from Nicola Marples and David Kelly, I developed the ideas, designed the study, interpreted the results, and wrote the chapter. To carry out the work required, I recruited and coordinated a team of collaborators drawn from relevant research groups in multiple Irish universities: Kevin Healy, Caroline McKeon, Darren O'Connell, Samuel Ross, Maude Baudraz, Cian White, Andrew Mooney, Samuel Preston, Floriane O'Keeffe, Hannah White, and Shane Somers. Together, this "Avipelago Team" and I developed the methodology and carried out the systematic review and data extraction. Caroline McKeon measured the geographic distances using island data that I compiled. Kevin Healy developed the statistical approach, based on techniques he had used in a previous collaborative macroecological project. The Bayesian statistical analyses presented in this chapter were performed by Kevin. Figure 7.1 was created by me, Figure 7.2 by Caroline and me, and Figures 7.3–7.5 by Kevin.

**Status:** This chapter presents preliminary results from a dataset which will continue to be analysed. After the thesis examination process, a final manuscript will be prepared and submitted to a biogeographic journal.

**Chapter Illustration:** Image used to introduce the ideas of the Avipelago project to collaborators.



## 7.1 Summary

This chapter presents a preliminary version of a macroecological study on dispersal and gene flow between islands. This study sought to investigate whether the ecological and behavioural traits of a given bird species influence gene flow, such that they affect the likelihood that island populations of that species will become isolated and speciate.

We carried out a systematic review to build a comprehensive library of papers which sequenced the ND2 gene of at least one island bird population. We assembled a dataset of location data for all these sequences, then calculated pairwise p-distances between all congeneric pairs. We used the minimum distances between islands as a measure of the physical barrier to dispersal. We used the genetic distance data as the response variable in a Bayesian model, with ecological, morphological, and behavioural trait data from other published sources as the independent variables, to investigate which traits affected the birds' ability to maintain gene flow across these barriers.

The dataset incorporated a total of 373 marine islands worldwide, including continental islands, oceanic islands, and continental fragments. These data were collected from 185 papers published between 1999 and 2020. Birds of 716 species were included.

We found a clear effect of water barriers on gene flow, as islands which were further apart had larger genetic distances. This effect was not uniform, as different island types exhibited different relationships between geographic distance and genetic distance. Morphological and behavioural features of the birds also affected genetic distance. For islands more than 100km apart, species with strong morphological dispersal ability (high Hand-Wing Index) exhibited lower genetic distances. Ground- and understorey-foraging species displayed stronger genetic divergence across most island distances, compared to species that forage in higher vegetation strata.

In keeping with classic biogeographic theory dating from when Wallace first drew his Line, we found that water barriers between islands pose significant dispersal barriers to birds, such that wider and more permanent stretches of water induce greater genetic divergence. The species which are least able to cross these barriers, due to their morphology or behaviour, are most likely to speciate.

## 7.2 Introduction

The process of dispersal, or “permanent movement away from an origin and long-term settlement at a new location” (Lowe and McPeck 2014), is of fundamental importance in evolution and biogeography. Since the very beginning, evolutionary biologists have seen the connection between the ability of a species to move across the landscape and its evolutionary trajectory, as when Darwin observed that birds are more likely than terrestrial vertebrates to colonise isolated islands, there evolving into new species in isolation (Darwin 1859). Indeed, the knowledge that certain bird species will travel farther than others pre-dates evolutionary biology entirely, and is found in the earliest surviving works of natural history (Aristotle c. 350 B.C.E). In the 20<sup>th</sup> century, dispersal between populations became a central idea in population genetics and the ensuing New Synthesis that revolutionised evolutionary biology (e.g. Dobzhansky 1940, Haldane 1948, Wright 1949). In this framework, populations accrue genetic differences in isolation, eventually leading to the formation of new species. This process of allopatric speciation was, and still is, recognised as the primary source of new species (Mayr 1959, Pigot and Tobias 2015). Populations separated by larger geographic distances will diverge more strongly, an effect known as isolation by distance (Wright 1943). Dispersal of individuals from the founder population into a newly isolated island population constitutes gene flow that will “swamp” the distinct evolution taking place on the island, so it is predicted that the less dispersal there is between populations the more likely speciation is to occur (Mayr 1942).

While developing the New Synthesis, Mayr recognised the role played by the ecology and behaviour of the species in question, which he termed “internal factors” influencing speciation. He gave the example of “far-ranging ducks” and “extremely localized geese” (Mayr 1942): As ducks will breed far from their birthplace (Lincoln 1933b, a), the duck species of North America maintain uniform populations across their ranges, while geese maintain close family systems (Heinroth 1911) and so have evolved into many geographic “races”. Therefore, internal factors can induce population structure even in a continental system, where the landscape itself did not impose such structure. Islands, on the other hand, represent clearly demarcated populations surrounded by barriers. The water barriers between islands are particularly important in restricting gene flow, being “harder” and less permeable than other kinds of biogeographic barriers such as lowland patches in between mountain habitat (Pujolar *et al.* 2022). Barriers on the mainland tend to reduce gene flow to a lesser extent, leading to only weak population structure (Menger *et al.* 2018). The distributions of birds across the islands of Southeast Asia were foundation stones in the work of Alfred Russel Wallace, who recognised them as records of evolutionary history (Wallace 1860, 1876, 1880). Many theories

concerning speciation were advanced using species limits of birds, especially those on islands (Mayr 1944, 1959, 1969, Diamond 1974, Mayr and Diamond 2001).

While island biogeography was developing as a distinct field, ringing (or banding) studies began to provide detailed information on how birds disperse (Paradis *et al.* 1998), and were used to estimate gene flow between populations in the absence of direct genetic evidence (Moore and Dolbeer 1989). Based on a review of such behavioural studies, Harris and Reed (2002) devised hypotheses that certain ecological and behavioural traits, including a skulking or understorey lifestyle, would inhibit birds in crossing barriers and so promote speciation. In evidence gathered from playback experiments, understorey foragers were found to be less likely to cross gaps in the forest than canopy feeders (St. Clair *et al.* 1998). Canopy feeders would be more accustomed to open, exposed areas, and might be better adapted for the risks of crossing barriers (Desrochers and Hannon 1997). Studies on second-growth forests have shown that understorey species are less likely to colonise isolated fragments or to maintain stable populations after fragmentation (Bradfer-Lawrence *et al.* 2018).

Molecular techniques (Sanger *et al.* 1977, Mullis *et al.* 1986) have become central in evolutionary biology (Haig *et al.* 2011, Whittaker *et al.* 2017). A growing number of DNA sequence phylogenies are being used to uncover the relationship within bird families (e.g. Cibois 2003, Nyári *et al.* 2009b, Andersen *et al.* 2015a, Cai *et al.* 2019) and to reconstruct the evolutionary history of birds in specific regions (e.g. Fabre *et al.* 2012, Pedersen *et al.* 2018, O'Connell *et al.* 2019b, O'Connell *et al.* 2019c). This creates an opportunity to test hypotheses about dispersal and gene flow directly. In the current biogeographic literature, explanations based on dispersal have been “in the ascendancy” (Whittaker *et al.* 2017). Several studies (Burney and Brumfield 2009, Smith *et al.* 2014) have sequenced South American bird populations on either side of barriers like the Andes and the Amazon, to uncover how speciation is affected by ecological and behavioural traits (Harris and Reed 2002).

While techniques like genomic sequencing are playing an increasingly large role in speciation research (Seehausen *et al.* 2014), they are not yet comparable in depth or breadth of sampling to studies based on Sanger sequencing of mitochondrial “barcoding” genes like cytochrome oxidase subunit 1 (COI) and NADH dehydrogenase 2 (ND2) (Hebert *et al.* 2004, Hebert *et al.* 2016, Wilson *et al.* 2016). In addition to its use in barcoding, many multilocus phylogeographic studies use ND2 along with other chosen mitochondrial

and nuclear genes (e.g. Linck *et al.* 2016, Pedersen *et al.* 2018, Cai *et al.* 2019, O'Connell *et al.* 2019b, O'Connell *et al.* 2019c, Pepke *et al.* 2019).

As widespread as the use of mitochondrial DNA (mtDNA) barcodes has been, it has come under some criticism (Rubinoff *et al.* 2006, Edwards and Bensch 2009). Evolutionary histories inferred from mtDNA can differ from those inferred from nuclear DNA (Rubinoff and Holland 2005, Phillimore *et al.* 2008), and patterns can be obscured by introgression and male-mediated gene flow (Toews and Brelsford 2012), though ND2 has shown a particularly high level of concordance with nuclear markers (Campillo *et al.* 2019). The fact that mtDNA is faster than nuclear DNA to complete lineage sorting and lose ancestral polymorphisms (Toews and Brelsford 2012) can also be an advantage, as it makes mtDNA more sensitive to population structure (Zink and Barrowclough 2008, Linck *et al.* 2016) and more informative of diversification on shallow time scales (Andersen *et al.* 2015b, O'Connell *et al.* 2019b).

Genetic studies are providing interesting insights into the impacts of dispersal on gene flow and speciation. Working in areas of continuous forest, Menger *et al.* (2018) found only weak evidence of fine-scale population structure in understorey-dwelling Amazonian birds, suggesting that they could disperse up to 10km. A different picture emerges in habitats separated by barriers. Burney and Brumfield (2009) found that populations of understorey-dwelling species on either side of the Andes, or of the Amazon or Madeira rivers, were significantly more genetically differentiated than were populations of canopy dwellers. In a later paper from the same research group, Smith *et al.* (2014) also found a strong link between foraging stratum and genetic divergence. These ideas have yet to be tested in the island context, however. In island systems, where water barriers are likely to be stronger in limiting gene flow, we can hypothesise that the differences between birds of different foraging strata may be even more dramatic. This chapter applies biogeographic concepts and methods, similar to those of the South American studies, to the island context.

As well as ecological traits, there has been an interest in investigating the effect of morphological differences between birds on their dispersal and gene flow. As wing length is a poor measure of dispersal ability (Dawideit *et al.* 2009), measures such as Hand-Wing Index (HWI) are preferred (Sheard *et al.* 2020). The HWI is an estimate of the shape of a bird's wing. Higher HWI scores indicate a more pointed wing, independent of overall size, thus better suited to long-distance dispersal (Kipp 1959). This index has come to be widely used as a proxy for morphological dispersal ability in macroecological studies (e.g. Weeks and

Claramunt 2014, Kennedy *et al.* 2016, Pigot *et al.* 2018). The HWI dataset compiled by Sheard *et al.* (2020) joins other large-scale datasets of species-level ecological traits (e.g. Wilman *et al.* 2014) that are increasingly being used by macroecologists to uncover the broad, general patterns underlying patterns of life on Earth (McGill 2019).

Our study used macroecological datasets, combined with the store of sequence data created for phylogenetic and evolutionary studies, to investigate dispersal and gene flow in island birds. We adapted hypotheses and methods from Burney and Brumfield (2009) and Smith *et al.* (2014), applying these to a wider geographic scale and to the uniquely fascinating evolutionary setting of the world's islands. Islands hold a disproportionate share of Earth's biodiversity (Cowie and Holland 2006), so we must understand the role of dispersal to islands if we are to focus conservation efforts to save evolutionarily important populations (Magnuson-Ford *et al.* 2010). We hypothesised that certain species-level ecological and behavioural traits would influence gene flow, such that island populations would be more or less likely to become genetically differentiated depending on these traits. We predicted that understory species, and birds with low HWI, would show the strongest genetic divergence at a given geographic distance.

## 7.3 Methods

### 7.3.1 Systematic review

Before starting our systematic review, we assessed the number of sequences of various avian genes available on the GenBank repository, as a proxy for the sample size that would be available for our study. We chose to base our measure of genetic divergence on the ND2 gene, as GenBank held a total of 54,000 ND2 sequences from birds, more than any other gene. As genetic divergence between populations relates to speciation (Harvey *et al.* 2017), the processes driving differentiation in this gene may also be driving the formation of new species over longer time periods.

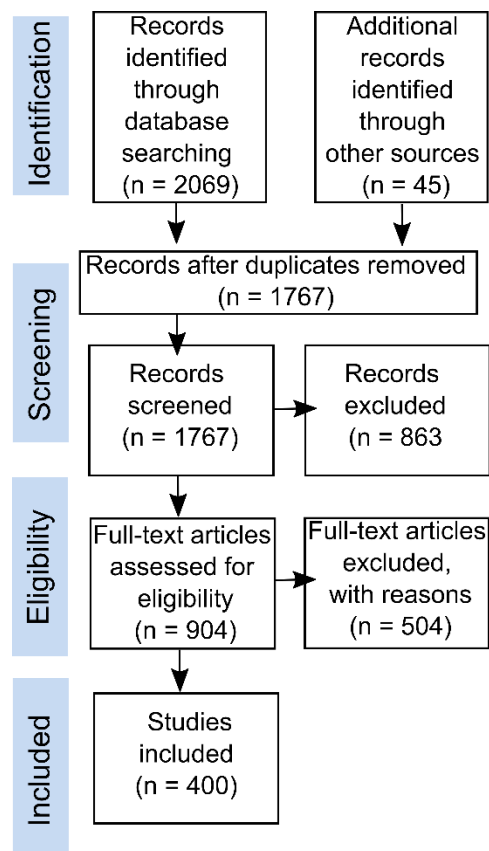
We carried out a systematic review (Figure 7.1) to collect our genetic data, searching for peer-reviewed studies which sequenced the ND2 gene in island birds and deposited their data in GenBank. We followed Weigelt and Kreft (2013) in defining an "island" as any landmass surrounded by ocean and smaller than Australia. We followed systematic review methods from Moher *et al.* (2009) to make our study transparent, reproducible and updatable (Gurevitch and Padilla 2004). We searched Google Scholar on the

29<sup>th</sup> of November 2019. As this database is very large, we used keywords to exclude its many parasitological studies, these being irrelevant to our question. We used the following search string for our Google Scholar search:

phylog\* AND (bird\* OR Aves OR avian OR ornith\*) AND (ND2 OR NADH2) AND GenBank AND (island\* OR archipelag\* OR insul\*) -parasite

We searched the Clarivate Web of Science database on the 3<sup>rd</sup> of December 2019. We used this simpler search string as there were fewer results:

(bird\* OR Aves OR avian OR ornith\*) AND (ND2 OR NADH2) AND (island\* OR archipelag\* OR insul\*) NOT parasite



**Figure 7.1.** PRISMA Flow diagram adapted from Moher *et al.* (2009), detailing our systematic review.

To check the effectiveness of our search, we prepared a list of 45 papers that we had read previously and knew to be relevant to our aims, to see if these would be identified by the review methods. After collating the papers from these three sources and removing duplicates, we were left with 1767 papers to review. 1730 of these came from Google Scholar, 30 from Web of Science, and 7 from the list of previously known papers. Therefore, 38 of our 45 (84%) previously known papers were found by the search method.

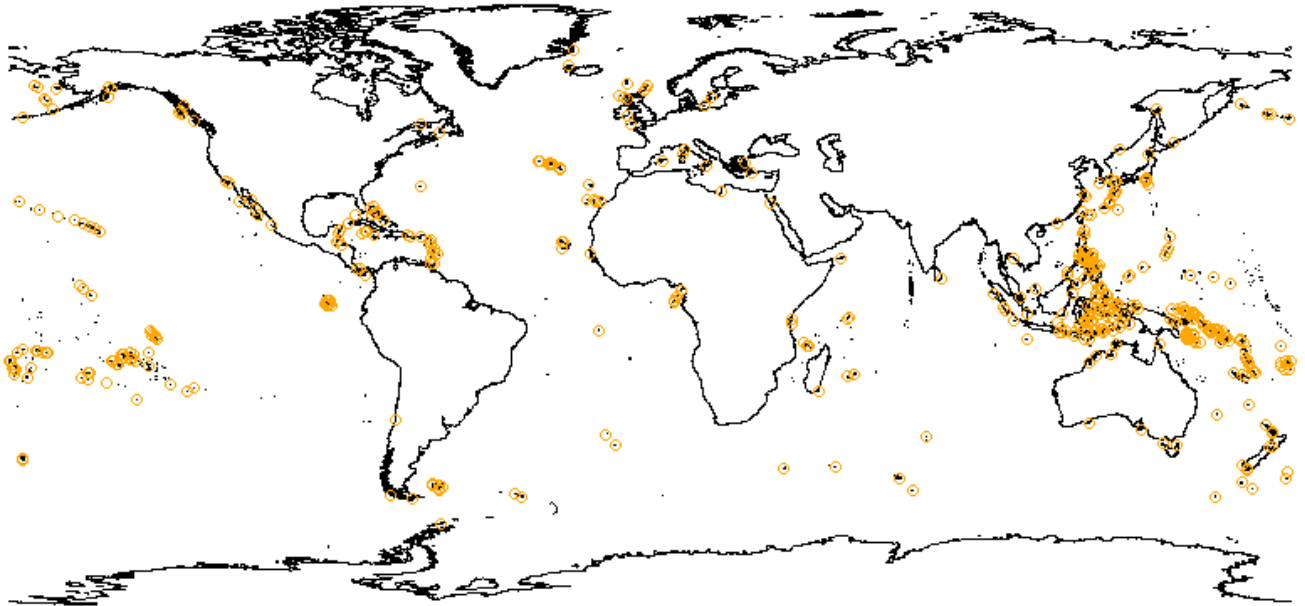
Following Moher *et al.* (2009), we decided which papers to include in the analysis in two stages, with the second stage using stricter criteria than the first. We used the website [sysrev.com](https://sysrev.com) for this process. The first stage used article titles and abstracts to eliminate papers that did not study birds or had no genetic component. Each record was assessed by two reviewers in the first stage to account for potential ambiguity (as titles and abstracts provide less information than full papers), with FÓM making the final decision in cases of disagreement. We excluded 863 papers during this stage, with details available here: <https://sysrev.com/u/1225/p/20656>.

In the second stage we used the full text of articles to create our final list of eligible papers. In this stage we accepted only papers that were published or accepted and in press (we excluded theses and pre-prints), by researchers who provided original ND2 sequence data (accepting either specific ND2 sequencing or full mitogenomes) from at least one bird from a specified island. Seabirds and migratory species were included only if sampled at their breeding location. Each record was assessed by one reviewer in the second stage, as they had access to more information than in the first stage. Again, uncertain cases were verified by FÓM. We also excluded papers that were not published in English. This is a potential source of bias in meta-analyses (Konno *et al.* 2020), but as our systematic review did not involve a meta-analysis of this kind, and was by necessity reliant on an English-language data repository (GenBank), we felt that this exclusion was unlikely to be detrimental. At the end of the systematic review, we had 400 eligible papers (Table S7.1).

### 7.3.2 Data extraction

We divided the 400 papers between our reviewers and used a standardised spreadsheet to extract relevant data from them. We recorded the GenBank accession number of each ND2 sequence used in the paper, noting when these were reported as originating in a different study. For each paper, we collected details on

every sequence that came from an island and every mainland sequence that belonged to the same species or genus as an island sequence. These mainland sequences were included so that mainland-island comparisons could be drawn. This approach gave us anywhere between 1 and 189 sequences from each paper. We noted the species name associated with each sequence and the island on which it was sampled or collected (Figure 7.2). When the paper itself did not specify the island, we searched for this information in the online supplementary information, the sequence’s GenBank page, and the museum’s online database, as applicable. Sequences without locality information, or those which failed to identify the island on which the sample was collected, could not be used. We treated certain island groups as single islands, where the individual islands were very close together such that biologists did not distinguish between them. Andros in the Bahamas and Aru off Papua New Guinea were examples of island groups treated as single islands in this way. We gathered collection ID numbers for the sequences that came from museum specimens, and isolate or sample numbers for those that were unvouchered.



**Figure 7.2.** Map of the world. Islands represented by sequences in the Avipelago database are highlighted with orange circles.

As the sequences had been published at various times by authors using different taxonomies, we used the package “taxalight” (Boettiger and Norman 2021) in R version 4.1.1 (R Core Team 2021) to standardise the species names according to the Integrated Taxonomic Information System (ITIS 2020).



Sequences were paired up based on their ITIS name, creating a dataset where each line represented a pair of sequences from the same species or genus.

We used Geneious Prime version 2021.2.2 (Biomatters 2021) to download and align all of the sequences associated with our list of accession numbers. While the ND2 gene is usually around 1041bp long, we decided to base our genetic distances on an alignment of 1000bp in order to maximise our sample size and its geographic coverage. Sequences of less than 1000bp or with gaps in the read (i.e. multiple bases in the sequence represented by X) were omitted at this point. We then exported the pairwise p-distances (proportion distances, i.e. the proportion of sites in each sequence that were different) from Geneious and fed it in to R for modelling as the dependent variable.

### 7.3.3 Geographic data

We collected data on the area of each island and its geological origin (Table S7.2) from the literature, where available, or by measuring the area using satellite imagery and the MyMaps function on Google Maps, if necessary. Island area is a key factor in classic island biogeography models (MacArthur and Wilson 1963, 1967).

We assigned each island to one of three geological types. “Continental islands” (or land-bridge islands) for those sitting on shallow shelves alongside larger landmasses, to which they were connected by land during the Pleistocene glaciations. “Oceanic islands” were those that originated in volcanism or the uplift of coral, and may have been connected to one another but not to any larger landmasses. “Continental fragments” were those consisting of small pieces of continental lithosphere, including microcontinental terranes like Sulawesi.

This classification of islands into three types dates back to the foundation of biogeography. Wallace (1880) divided islands in this way, though he used the name “ancient continental island” in place of continental fragment, and had difficulty classifying islands like Sulawesi and New Zealand, which he termed “anomalous”. It is worth noting that Wallace lived in an era before plate tectonics, and as understanding of Earth’s geological history has improved, so biogeographers have been replacing his scheme with one that is more accurate and detailed (Ali 2017, 2018). However, it was not feasible to interrogate the geological

literature for this level of detail on all of our islands, this literature being in any case lacking for some of the more obscure islands. Therefore we settled on the three-category Wallace (1880) scheme as an acceptable representation of island isolation in space and time (Flantua *et al.* 2020).

We used the R packages `rnaturalearth` (South 2017) to view detailed, high-resolution polygons of the Earth's landmasses from the NaturalEarth dataset ([naturalearthdata.com](http://naturalearthdata.com)). We then identified which polygons represented the islands where our sequences were sampled. Many islands weren't pinpointed precisely in the papers, or were identified using historical names in museum databases, so we searched the literature to ensure that we identified each island accurately and standardised their names. Some islands, particularly smaller ones, were absent from the NaturalEarth dataset and so for these we drew our own polygons using coordinates we obtained from satellite imagery. We then used the package `gdistance` (van Etten 2017) to calculate the minimum distance between each pair of islands, i.e. the width of the water barrier separating these islands' populations. To account for the fact that some of these water barriers were broken up by other islands, we also calculated a "minimum stepping stone distance" for each pair. This was based on the stepping stone isolation measure tested by Weigelt and Kreft (2013), which they found to be a good predictor of plant species richness and which they suggested would be particularly relevant for birds. To calculate this, Weigelt and Kreft (2013) found the shortest line between an island and the nearest continent, then assigned a "cost distance" based on this line, such that crossing water was taken to be more "costly" than crossing land. We modified this approach to give the stepping-stone distance between two islands and increased the cost assigned to crossing water to be ten times that of crossing land. We reasoned that the difference in "cost" would be larger for birds than plants, as many birds are known for the stark contrast between how freely they fly across land and how reluctant they are to cross water barriers (e.g. Mayr 1969, Diamond 1981).

### 7.3.4 Ecological data

We obtained species-level data on foraging stratum from the EltonTraits database (Wilman *et al.* 2014). These foraging stratum data are presented in five columns, each one representing the percentage of time which the species spends in any one stratum: ground, understorey, mid-high, canopy, and aerial. It also assigns percentages for time spent in water below and around the surf. We used these latter two columns to exclude species that spent over 50% of their time in the water, in order to focus on terrestrial species. We

then derived a “stratum index” from the five foraging stratum columns, by multiplying and then adding or subtracting their values according to the following formula:

$$\begin{aligned} \text{Stratum index} &= 2 * \text{percentage of time spent aerially} \\ &+ 1 * \text{percentage of time spent in canopy} \\ &+ 0 * \text{percentage of time spent in mid-high stratum} \\ &- 1 * \text{percentage of time spent in understory} \\ &- 2 * \text{percentage of time spent on ground} \end{aligned}$$

This collapsed the stratum data into a single continuous variable, and assisted modelling by avoiding multiple categorical variables. A positive stratum index indicates that the species lives higher in the habitat, which is expected to lead to increased dispersal. Examples of species with strongly positive stratum indices included *Collocalia esculenta* (Glossy Swiftlet, stratum index of 180) and *Aerodramus leucophaeus* (Tahiti Swiftlet, stratum index of 180). A negative stratum index indicates that the species lives below the midstorey. Such species are expected to show an inhibited dispersal. Strongly negative dispersal indices were assigned to species like *Gallus varius* (Green Junglefowl, stratum index of -200) and *Troglodytes troglodytes* (Eurasian Wren, stratum index of -150). Due to a quirk in the recording of feeding behaviour by Wilman *et al.* (2014), raptors which take their prey from the ground were also assigned negative stratum indices, such as *Falco newtoni* (Malagasy Kestrel, stratum index of -150) and *Milvus milvus* (Red Kite, stratum index of -200). There were comparatively few such species in our dataset, however.

For a direct measure of physical dispersal ability, we obtained data on Hand-Wing Index (HWI) for each species from Sheard *et al.* (2020).

### 7.3.4 Statistical modelling

For this study we adapted a Bayesian approach developed by KH for Csergő *et al.* (Preprint), fitting Bayesian phylogenetic mixed models using the package MCMCgImm (Hadfield 2010). This allowed us to incorporate

multiple rows of pairs with the same individual DNA sequences in them while avoiding pseudoreplication. Similar to the use of bootstraps in phylogenetic reconstruction (Felsenstein 1985), this approach creates 100 randomised versions of the dataset, with each sequence included only once in each dataset (thus avoiding pseudoreplication), and runs a separate model for each iteration. An overall model then analyses which relationships were consistent throughout the 100 iterations. Our model incorporated the Jetz *et al.* (2012) global bird phylogeny in order to correct for the phylogenetic relationship between the birds. Where multiple individuals of the same species had been sequenced on the same island, one sequence was selected at random to represent the population. To account for the fact that some islands (and papers) contributed more sequences than others, we included “island” as a random effect in the model. This avoided any potential bias associated with favoured study sites.

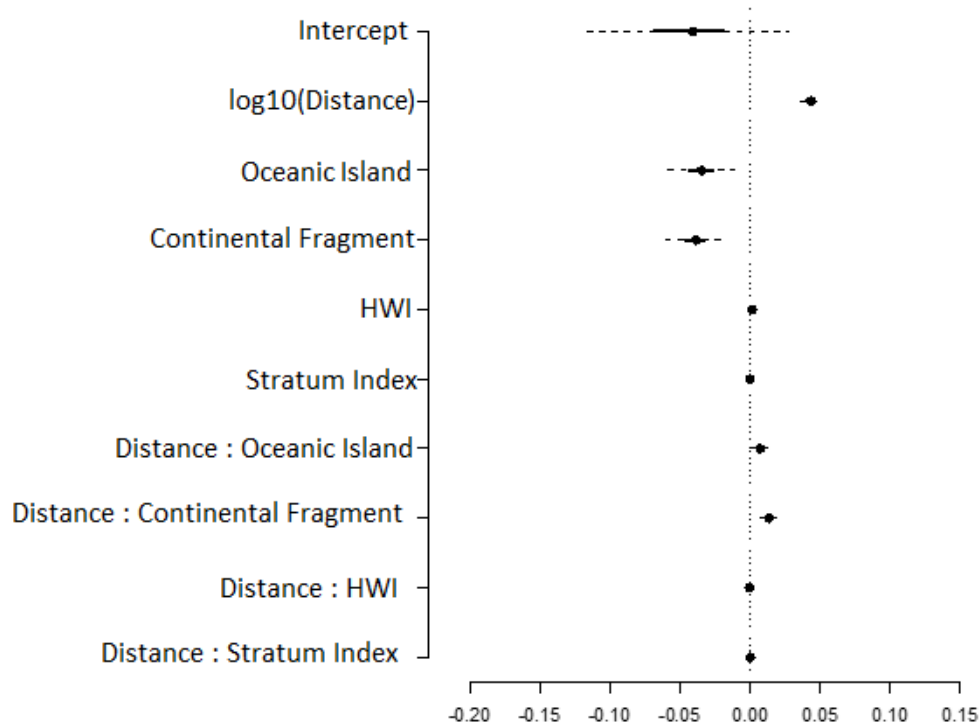
We were interested in the traits of a species that would affect dispersal and gene flow between populations of that species. We therefore designed a model to test for the effect of geographic distance, island type, HWI, and stratum index, on the genetic divergence between two populations. We also included interaction terms between geographic distance and the other traits, to see if two populations which were a given distance from one another would be more or less genetically distinct, depending on their ecological traits. As the HWI and stratum data were assigned at the species level, for genus-level comparisons we used the mean value of the two species.

For this preliminary version of the study, we used the simplest geographic distance measure (minimum distance in kilometres), in  $\text{Log}_{10}$  form. We aim to revisit the stepping-stone distance measure in a later analysis. Later models will also consider data we have collected but not incorporated yet, such as island area, and data which we have the means to measure but have not yet collected, such as distance from the nearest continent.

## 7.4 Results

Figure 7.3 shows the effects of the different independent variables and their interactions on genetic p-distance. We found a clear isolation-by-distance effect in our data: Islands with higher geographic distances between them, or (alternatively), wider water barriers separating them, exhibited stronger genetic divergence. Populations on oceanic islands and continental fragments exhibited lower genetic divergences

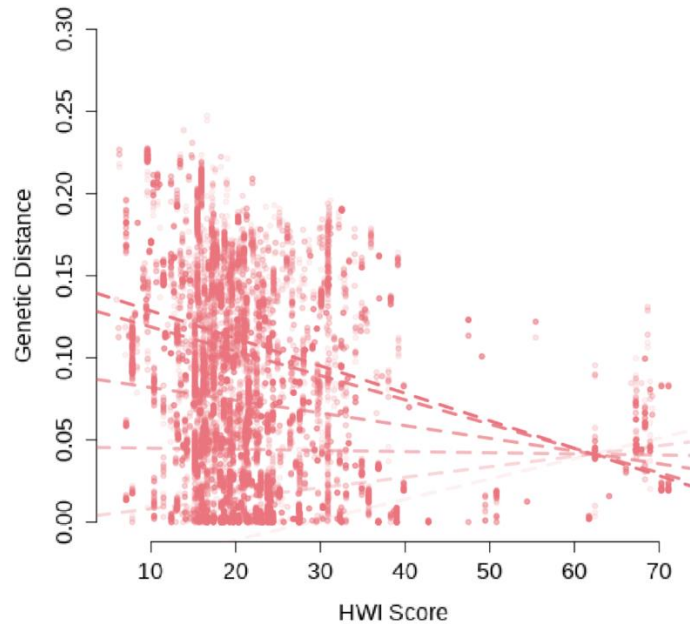
overall, but these variables had a positive interaction with geographic distance. This means that the isolation-by-distance effect was stronger in oceanic islands and continental fragments, compared to continental islands.



**Figure 7.3.** The combined posterior distributions across all 100 datasets for the main model. The labels on the left represent independent variables from the model, along with the overall intercept. The black circles represent the effect of these variables on genetic p-distance (the independent variable). The farther the black circle is from the dotted line (which represents “no effect”), the stronger the effect of this variable on genetic p-distance. “log10(Distance)” is the log in base 10 of the minimum distance between islands, the effect of “Oceanic Island” and “Continental Fragment” are tested relative to continental islands, HWI is Hand-Wing Index, and the last four variables are the interactions between geographic distance and the other variables. Continental islands were chosen as the “baseline” as they are expected to be the least isolated. Random effects are not shown here.

Our metric of physical dispersal ability, HWI, affected the relationship between genetic distance and geographic difference in different ways at different distances (Figure 7.4). In island pairs with narrow water barriers between them, the relationship between HWI and genetic divergence was positive but very weak, indicating no clear difference between birds with weaker and stronger dispersal abilities. However, for island pairs with distances above 100km there was a stronger, negative relationship between HWI and genetic

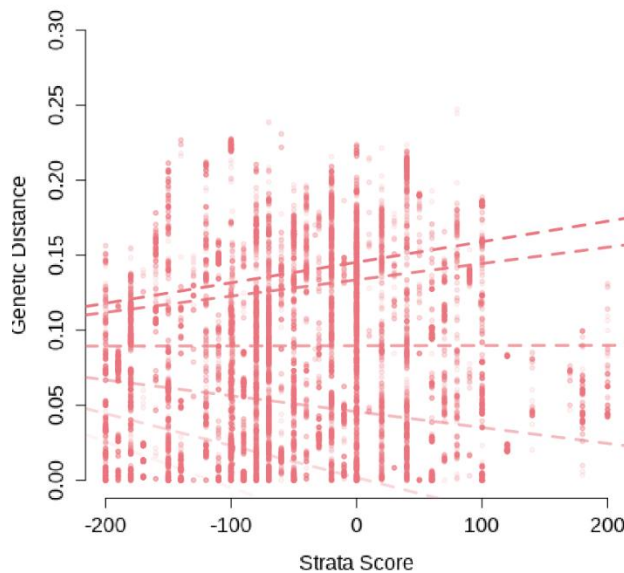
distance. In other words, on any given pair of islands separated by a water barrier of 100km or more, populations of birds with weaker dispersal ability were more genetically differentiated. This was in keeping with our predictions.



**Figure 7.4.** Plot showing the relationship between Hand-Wing Index (HWI) and genetic p-distance.

The palest line represents the relationship on islands 1.4km apart from each other (the lowest distance in the dataset), the darkest line the relationship on islands 18479km apart (the longest distance in the dataset). Between those two lines are (in order of increasing darkness), the relationship at distances of 10km, 100km, 1000km, and 10,000km.

The effect of foraging stratum also varied with geographic distance (Figure 7.5). At distances below 1000km there was a negative relationship between stratum index and genetic distance, as we predicted, i.e. ground-dwelling and understorey species were more genetically divergent than species from higher strata. There was a positive relationship between stratum index and genetic distance on islands more than 1000km apart, however. This may be due to the treatment of birds of prey as “understorey” species, since raptors are generally wide-ranging birds.



**Figure 7.5.** Plot showing the relationship between stratum index and genetic p-distance.

The palest line represents the relationship on islands 1.4km apart from each other (the lowest distance in the dataset), the darkest line the relationship on islands 18479km apart (the longest distance in the dataset). Between those two lines are (in order of increasing darkness), the relationship at distances of 10km, 100km, 1000km, and 10,000km.

## 7.5 Discussion

Our model successfully captured the isolating effect that water barriers between islands have on bird populations, showing that wider water barriers between population pairs are associated with increased genetic divergence. Biogeographers have long been interested in the fact that many birds are unwilling to fly across water. This phenomenon has been studied mostly in tropical species, with Mayr (1969) remarking that “Most tropical birds are highly sedentary and respect water barriers to a high degree”. Entire families of birds are absent from oceanic islands in the Neotropics (Terborgh 1975), and many of New Guinea’s understorey birds won’t cross even narrow water barriers (Diamond 1972). Diamond (1971) attributed the sedentary lifestyle of tropical birds to the relative climatic stability of their habitats. It should be noted here that, while temperate birds may be more dispersive than their tropical counterparts, many are still subject to isolation by water barriers (Beddall 1963, Harris and Reed 2002). The effect of water barriers is at least partially psychological, as many birds will readily fly across land but not water (e.g. Mayr 1969, Diamond 1981). This behaviour may have arisen because many birds make poor colonisers of islands due to their low reproductive potential (Diamond 1972).

The negative effect of the “oceanic island” variable on genetic divergence was contrary to the expectation that these islands should be more isolated than continental islands. While oceanic islands are famously isolated from continents (Wallace 1880), islands in many oceanic archipelagos were connected to one another during the Pleistocene glaciations (Weigelt *et al.* 2016). This is relevant to this analysis as it compared populations on oceanic islands to one another, rather than to populations on the mainland. This might be why the “oceanic island” variable had a negative effect on genetic divergence but a positive interaction with geographic distance, as more distant oceanic islands would not have had land bridges with each other.

The ecology and morphology of the birds themselves are also a key factor in their isolation. Birds with a skulking, low-stratum lifestyle or low HWI are less inclined to disperse across barriers. Populations of these species exhibit increased genetic divergence due to reduced gene flow. This mirrors the findings of previous work on populations living among rivers and mountains in South America (Burney and Brumfield 2009, Smith *et al.* 2014) and validates the hypothesis of Harris and Reed (2002), that an understorey lifestyle acts as a behavioural barrier to dispersal.

The results to date are encouraging. Islands are among the world’s most important threatened hotspots of biodiversity (Bellard *et al.* 2014) and have long been pivotal in the study of speciation (Wallace 1880, Mayr and Diamond 2001). This study demonstrates that water barriers between islands are key drivers of evolution, with the exact relationship differing based on the geological origin of the islands and the traits of the species in question. As our analyses evolve, we will continue to learn more about how evolution has generated these beautiful, fragile collections of living things.

## 7.6 Future work

We intend to make some additions and changes to our model as this research continues. While the current effect size estimates for both the stratum index and HWI are small, this relates to the difference in the range of values for these parameters compared to geographic distance. For example, there are 400 possible values for the stratum index (-200 to 200). The model calculated an effect size estimate of -0.0004 for the stratum index, which over the whole range of stratum index values adds up to  $-0.0004 * 400 = -0.13$ . This is



comparable to the isolation-by-distance effect size ( $0.04 * 4.12 = 0.18$ ). We will look into the use of scaling to convey the effect of these parameters more accurately.

Despite their isolation, islands do not exist in a vacuum. Continents play a role in the ecology and evolution of island biotas, and are particularly important to continental islands, to which they were connected in the geologically recent past (Flantua *et al.* 2020). During the course of our data extraction, we also collected information on sequences from continental populations when these were presented by the authors. We intend to incorporate genetic distances between continental and island populations of a genus or species into a later version of our model, along with the minimum distance between islands and the nearest continent. Both of these forms of data can be collected using the methods we have already established for inter-island pairs. We expect that continental data will help to clarify the geological aspects of isolation and the differences between the continental islands, oceanic islands, and continental fragments. We will run the model on a version of the dataset that omits raptors, to see if this changes the effect of foraging stratum on long-distance pairs. We will include “archipelago” as a random effect, to control for the fact that some of our oceanic islands are in the same archipelago, and thus will have been connected to one another by land bridges.

## Chapter 8 - General Discussion

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*“There are no more islands. Yet there is a need for them. In order to understand the world, one has to turn away from it on occasion...”*

– Albert Camus (1939)

**Chapter Illustration:** Detail from the cover of Wallace (1876), depicting the super-endemic avifauna of Madagascar.

Many biologists have used islands as “natural laboratories”, where they can work to understand the world and the evolution of life by studying it in a specific, isolated set of circumstances (Whittaker *et al.* 2017). The field of biogeography, in particular, was developed largely on islands, where the distribution of organisms was used to discover how new species arise and disperse across the world (Whittaker and Fernández-Palacios 2007). Such studies are limited by our knowledge of biodiversity (Hortal *et al.* 2015). We have not yet named all of the world’s species (Linnean shortfalls) and we have imprecise knowledge of the distributions of many species (Wallacean shortfalls).

As laid out in the General Introduction, this thesis had three main aims:

1. To help address some Linnean and Wallacean shortfalls in our knowledge of island birds, as any more complex theories rely on accurate knowledge of the biota itself.
2. To explore the population structure of birds in the important biodiversity hotspot of Sulawesi and its surrounding regions, supplying insights into evolutionary processes.
3. To use the patterns thus uncovered to refine certain concepts from biogeography, in particular our understanding of how differences in the dispersal ability of birds affect gene flow. This, in turn, drives patterns of speciation and thus the generation of biodiversity.

Each of these aims builds on the previous one: knowing the number of species and their distributions allows us to study population structure, which in turn can be used to test biogeographic ideas. This General Discussion will outline how the preceding data chapters have advanced these aims, as well as recommending future avenues of research.

## 8.1 Wallacean and Linnean shortfalls

Despite the importance of tropical faunas to biodiversity and evolutionary biology, basic knowledge of these faunas is often lacking (Reboredo Segovia *et al.* 2020), including accurate knowledge of species distributions (Hughes *et al.* 2021). Addressing the Wallacean and Linnean shortfalls requires cooperative and coordinated action from biologists all over the world (Raven and Wilson 1992). While working on this thesis I have contributed distribution data which has addressed several Wallacean shortfalls, including Chapter 2 of this thesis (published as Ó Marcaigh *et al.* 2020) and several other papers (O’Connell *et al.* 2017, O’Connell *et al.* 2019d, Palfrey *et al.* 2019). Taken together, this work has provided the first recent, detailed species inventories of the Ankobohobo Wetland IBA and Mariarano-Matsedroy forest fragments in Madagascar; Kabaena, Wawonii, and Muna islands in Indonesia; and a portion of the Southeast Sulawesi peninsula. Such work is particularly important when threatened and endemic species are involved: for example, information from Ó Marcaigh *et al.* (2020) has been incorporated into a reference work on the endangered Humblot’s Heron (Billerman *et al.* 2022) and a WWF publication on eagles (Probst and Pichler 2021).

Wallacean shortfalls pose a particular challenge to biogeographers, as it is hard to divine the drivers of species distributions patterns when those distributions are not known (Whittaker *et al.* 2005). Linnean

shortfalls pose a challenge to all biologists, as the species is “the basic unit of evolutionary biology” (Mayr 1982). Taxonomists have blamed Linnean shortfalls on the lack of support that their discipline receives (Engel *et al.* 2021). Indeed, there has been debate as to the feasibility of naming Earth’s species before a large portion of them go extinct (Costello *et al.* 2013, Laurance 2013). In total, this thesis has identified seven potentially undescribed species, some of which will require further sampling by other biologists before they can be formally named. Potential species requiring further sampling are the “Melanesian Island Monarch” (Chapter 5), the “*aurora* Olive-backed Sunbird”, and the “New Guinea Black Sunbird” (Chapter 6). Meanwhile Chapter 6 has confirmed the species status of the Wakatobi Sunbird using integrative taxonomy, and contributed genetic evidence to the three-way split of the “Sahul Sunbird”, “Garden Sunbird”, and “Ornate Sunbird” (Eaton *et al.* 2021). Another paper I co-authored, while working on this thesis, identified two new white-eye species in the Wakatobi Islands (O’Connell *et al.* 2019c)

Addressing Linnean shortfalls requires evaluating diversity at, above, and below the species level. It is important to understand the diversity within species as well as the number of species, as such diversity must arise before speciation can occur (Dobzhansky 1940). The treatment of intraspecific diversity has been subject to debate (Wiens *et al.* 1982), with the subspecies (Esper 1781, Mayr 1963) persisting as a taxonomic rank despite criticism of how the concept is applied and the concept itself being labelled “arbitrary” (Patten 2015). Some conservationists have instead used the concept of “evolutionarily significant units” (ESUs) to examine the adaptive variation in a species (Moritz 1994), when this is not adequately captured by taxonomy (Ryder 1986). My approach to this issue has been informed by recommendations that subspecies are most useful on islands, in the tropics (Phillimore and Owens 2006), and when they are based on multiple lines of evidence (Wiens *et al.* 1982). This thesis has identified seven populations that may represent unrecognised subspecies: the Sulawesi Babbler on Southeast Sulawesi, Muna, and Buton (formerly named *improbatum*), the Sulawesi Babbler on Kabaena, the Sulawesi Babbler on Wawonii (All in Chapter 4), the Karkar population of Island Monarch (Chapter 5), and the Menui populations of Island Monarch (Chapter 5) and Black Sunbird (Chapter 6). These populations exhibit a degree of evolutionary divergence from the rest of their species, which warrants consideration by biologists and conservationists whether they are designated as subspecies or ESUs (Moritz 1994).

Along with the subspecies, the species too has generated its own controversies. To answer the question “how many species are there?” raises the thorny issue of what constitutes a species. Some biologists maintain that species are essentially arbitrary and exist only *a posteriori* to our definition of them

(Raposo *et al.* 2021). Having by then spent several decades studying species and speciation, Mayr (1982) remarked wryly that “There is probably no other concept in biology that has remained so consistently controversial as the species concept”. By the turn of the century, however, de Queiroz (1999) argued that this problem had been solved and suggested that each biologist can select species properties that are most relevant to the question being addressed. My approach has been to accept the argument of de Queiroz (1999, 2007) that species do exist in nature, independent of our concept of them, and that the species controversy turns on how we delimit these real entities. This thesis has thus worked to identify which populations represent evolutionarily independent lineages, quantifying their diversity and the divergence between them. In this thesis I applied the standard integrative methodology accepted in ornithology (Tobias *et al.* 2010), combined with genetic analysis to detect cryptic diversity (Rheindt and Ng 2021, Tobias *et al.* 2021). DNA sequencing and integrative taxonomy are vital approaches, if we are to ascertain how many species exist (addressing Linnean shortfalls). Similarly, field surveys which sample and describe where populations are distributed are essential to address Wallacean shortfalls.

## 8.2 Population structure around Sulawesi

Sulawesi is noted as a region of high bird endemism (Stattersfield *et al.* 1998). One reason to study the number, diversity, and distribution of species is to give an understanding of how this endemic fauna is shaped by evolutionary processes. Taken in combination, efforts to address the Linnean and Wallacean shortfalls will create a picture of population structure, as they involve measuring the divergence between and within populations. Such close examination can change our understanding of a biota quite radically: elsewhere in the Indo-Pacific, the Philippines were discovered to have a much higher level of endemism than had been thought, once cryptic species were recognised (Lohman *et al.* 2010). This thesis has shown that the endemism of the Sulawesi region is still underestimated.

This thesis has highlighted that some of the islands off Sulawesi are particularly important in generating biodiversity. Identifying islands with unique biotas has long been a priority of biogeographers (Wallace 1880) and has only grown in importance as the world’s islands have become more threatened (Whittaker *et al.* 2017). The work outlined in Chapters 4, 5, and 6, has helped to give a better understanding of evolutionarily divergent populations on islands like Kabaena, Wawonii, Menui, and the Wakatobi Islands, showing how these are related to each other and how they fit in the wider Sulawesi and Indo-Pacific biota.

Our understanding of endemism and population structure must be informed by the geology of the regions we study. Sulawesi's peninsulae originated as separate islands (Michaux and Ung 2021), and their biotas show differences from one another, to the point that even bird species can diverge from one peninsula to another (Chapter 4). The Wakatobi Islands have always been separated by water from Sulawesi and all other larger landmasses (Nugraha and Hall 2018), and so they would be expected to hold divergent fauna, like other "oceanic" islands around Sulawesi (Rheindt *et al.* 2020). The isolated location of the Wakatobi Islands is reflected in the unique maritime traditions of the people who live there (Mansyur *et al.* 2021). Nevertheless, a lack of ornithological attention resulted in their endemism being underrated until recently (O'Connell *et al.* 2020b). Chapter 6 reveals that the "Wakatobi Sunbird", treated as a subspecies of the Olive-backed Sunbird (*Cinnyris jugularis infrenatus*; Gill *et al.* 2022), is actually an endemic species. The "Wakatobi Sunbird" was first described as a species by Hartert (1903), along with the Wakatobi Flowerpecker and Wakatobi White-eye. Like the sunbird, these were both demoted to subspecies, before modern studies, using integrative techniques, reclassified them as full species (Kelly *et al.* 2014, O'Connell *et al.* 2019c). One advantage of having a taxonomic rank below species is that it keeps divergent populations like these from being "lost" after they are demoted from species level (i.e. 'lumped'). This allows for future consideration when new techniques become available. The range of the *Cinnyris jugularis infrenatus* subspecies was misrepresented in the literature until recently (Kelly and Marples 2011), an example of a Wallacean shortfall affecting our understanding of population structure: it is difficult to ascertain the level of a population's evolutionary independence when you do not know where to look for it.

Sulawesi's land-bridge islands were connected to the mainland in recent evolutionary history, and so we would expect their biotas to be almost identical to those of the mainland (Wallace 1887). Indeed, many bird species exhibit a genetically continuous population between these islands, such as the monarchs and sunbirds (see Chapters 5 and 6), and also the white-eyes (O'Connell *et al.* 2019c). Crucially, though, some birds have diverged between Sulawesi and certain of its land-bridge islands, namely the babblers investigated in Chapters 3 and 4 and the Red-backed Thrush (Robinson-Dean *et al.* 2002). This may relate to their dispersal ability, as *Pellorneum* babblers and *Geokichla* thrushes share an understorey lifestyle known to impede dispersal and gene flow (Harris and Reed 2002, Burney and Brumfield 2009, Smith *et al.* 2014). Kabaena and Wawonii stand out in Chapter 4 as holding the most divergent bird populations of the land-bridge islands. Potentially, this might be related to their ultramafic geology (Galey *et al.* 2017), which is associated with plant endemism (Anacker 2014). Thus, as well as the broad patterns of isolation and connection associated with different island types, specific characters of their geology can shape the selective environment and influence population structure.

Like Kabaena and Wawonii, Menui is another island where some birds have diverged and not others. Even within one family, the Black Sunbird has produced a divergent population on Menui while the Olive-backed Sunbird has not (Chapter 6). Menui's geological relationship to the Sulawesi mainland is complex. Older reconstructions placed Menui on a relatively shallow continental shelf with the mainland of Southeast Sulawesi, like the land-bridge islands (Hall 2002). On newer maps, this has been replaced by a patchy, thin band of carbonate platforms, like the ones around the Wakatobi Islands, running between Menui and Southeast Sulawesi (Hall 2013). The Tolo Thrust runs near Menui (Hall 2012), leading to complex patterns of deformation in the region's rock (Titu-Eki and Hall 2020). The ultimate outcome is that there does not seem to have been a land bridge between Menui and the mainland during the Pleistocene glaciations (Nugraha and Hall 2018). The avifauna of Menui is more "oceanic" in its composition, reflected by the presence of Island Monarchs and the absence of Pale-blue Monarchs (Chapter 5). The difference in speciation patterns between Menui's sunbird populations may reflect fine ecological differences between the sunbird species, which have not been fully described, or some element of chance.

The population structure of an island system does not arise purely deterministically from geology and ecology, but will result partly from chance. Island colonisation, in particular, has been described as a stochastic process (Simberloff 1978). In this thesis, the island of Runduma presents an illustrative case. Most of the bird species on Runduma are commensal with humans, or island specialists like the Island Monarch (Chapter 5). The Olive-backed Sunbird and the Lemon-bellied White-eye are among the few species occurring there which do not fit either of these categories. These two populations have very different histories, however, as Runduma's sunbird population is genetically continuous with that of the Wakatobi Islands (Chapter 6), while its white-eyes have their closest relatives on mainland Southeast Sulawesi (O'Connell *et al.* 2019c). Nevertheless, both of these small birds have established themselves on an island that is just 5.5 km<sup>2</sup> in area, after crossing more than 50 km of open sea in opposite directions (O'Connell *et al.* 2020b).

Using Sulawesi as a study system allows consideration of the history of dispersal on both small and large geographic scales, as different birds followed different colonisation routes across Wallacea. The region's island chains provide scope for many such routes: indeed, Wallacea was the site of the first dispersal and radiation of the songbird lineage (Moyle *et al.* 2016). Many of the birds considered in this thesis have their closest relatives in Asia, west of Wallace's Line, including the Sulawesi Babbler (Chapters 3 and 4), Pale-blue

Monarch (Chapter 5), and both the *Cinnyris* and *Leptocoma* sunbirds (Chapter 6). They differ greatly in how far they have dispersed across the Indo-Pacific, however. The Sulawesi Babbler on Sulawesi and its land-bridge islands is the farthest east of all *Pellorneum* babblers, indeed it is the only *Pellorneum* species to be found east of Wallace's Line (Billerman *et al.* 2022), while the Olive-backed Sunbird presents a genetically similar population all the way from Sulawesi to Australia (Chapter 6). The Island Monarch (Chapter 5) is unique among the species dealt with here, in that its closest relatives are all found far to the east, around Melanesia or the Sahul Shelf or Micronesia. Indeed, the Island Monarch is the only representative of *Monarcha* west of Lydekker's Line (Billerman *et al.* 2022). These contrasts illustrate the position of Wallacea as a zone of transition between very distinct faunas, and as one of biogeography's foremost "natural laboratories".

The composition and population structure of the birds around Sulawesi and the Wakatobi Islands clearly mark this region as of great biogeographic interest. We recommend the Sulawesi Endemic Bird Area (EBA) should be divided into several new EBAs, with Southeast Sulawesi (including its land-bridge islands) and the Wakatobi Islands recognised as distinct centres of endemism (Evans *et al.* 2003, Frantz *et al.* 2018, Michaux and Ung 2021). Menui and Runduma would be included in the Southeast Sulawesi and Wakatobi Islands EBAs, respectively, although their avifaunas are distinct in themselves and warrant further study.

My final point on population structure relates to methodology. Much has been made of the application of genomic, next-generation, and other advanced sequencing techniques to the study of speciation (Seehausen *et al.* 2014). The B10K Birds Project aims to sequence a representative genome for each extant bird species, as recognised by current taxonomy (Feng *et al.* 2020). This approach promises to settle many questions about the lower branches of the avian evolutionary tree, but it cannot reveal population structure or cryptic diversity within these species. Indeed, an indeterminate number of undescribed cryptic species will not be included at all. Sanger sequencing of mitochondrial DNA still has a role in allowing a broad overview of population structure, as its lower cost allows more populations to be sequenced, with a larger sample size, for any given budget. Traditional Sanger sequencing has particular advantages of its own, producing relatively long sequences using conserved primers with known genomic positions, with fewer errors compared to newer sequencing methods (Toews *et al.* 2016). Some of the species-level splits proposed by this thesis, such as that between the Black Sunbird in Wallacea and Papua, were not even suggested until this methodology was applied to them. This emphasises that much cryptic diversity surely remains to be discovered in this region. Many Indo-Pacific birds have specimens in museums



around the world, mostly collected before the second world war (Rheindt *et al.* 2020), to which no genetic methods have been applied at all. We recommend that as many Indo-Pacific birds as possible be sequenced using the methodology that allows the widest possible coverage. Any divisions suggested by DNA sequencing may be tested using integrative taxonomy, which can also be performed without expensive equipment (Tobias *et al.* 2010).

### 8.3 Dispersal, gene flow, and biogeography

The preceding sections have made one thing clear: although Sulawesi is already recognised for the endemism of its avifauna (Stattersfield *et al.* 1998), and Wallacea is known as a threatened biodiversity hotspot (Myers *et al.* 2000), the region's biodiversity is even higher than has been represented in the literature. After addressing Wallacean and Linnean shortfalls and assessing population structure, classic biogeographic theory can be tested against the patterns uncovered. The general conclusions from this work informed the Avipelago project (chapter 7), which took a broad view to determine general patterns in the speciation of island birds. Throughout this thesis, the interactions between dispersal, gene flow, and speciation were of particular interest. This section synthesises the overall findings.

Chapters 3 and 4 (published as Ó Marcaigh *et al.* 2021a, Ó Marcaigh *et al.* 2021b) examined an understory bird species (the Sulawesi Babbler) in a continental land-bridge island system. The land-bridge islands of Southeast Sulawesi formed a continuous landmass until rising sea levels disconnected them (Nugraha and Hall 2018). These studies thus amounted to examining whether those new water barriers disconnected the islands' bird populations as well or whether gene flow persists between them, i.e. have these populations been divided by vicariance (Whittaker and Fernández-Palacios 2007). Chapter 3 found that there are particular ecological pressures acting on babbler populations on the land-bridge islands, causing them to exhibit stronger sexual dimorphism than those on the mainland of Sulawesi. Chapter 4 looked at general evolutionary divergence of babblers, and it found that the Kabaena and Wawonii populations had diverged from the mainland Southeast Sulawesi population, but those on Muna and Buton had not. As all of these islands are subject to a similar degree of physical isolation, this difference suggests that while the ecological pressures of living on land-bridge islands can induce some morphological changes, these pressures are not enough alone to cause the divergence seen on Kabaena and Wawonii. Additional selection pressures, like those induced by ultramafic soils, may be necessary for land-bridge populations to diverge acoustically and genetically as well as morphologically. Overall, Chapters 3 and 4 indicate that continental island

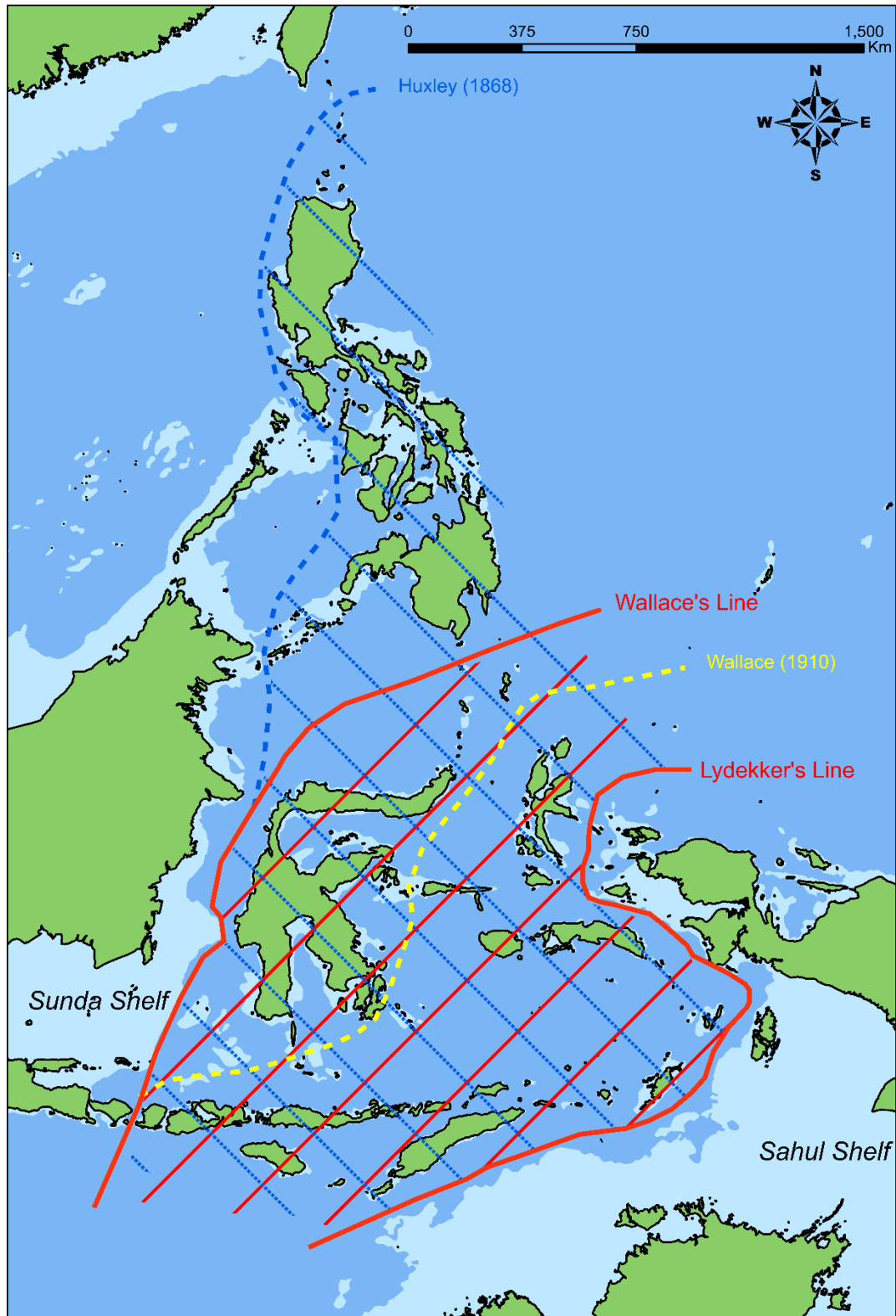
populations can be important in the evolution of a species. This runs counter to classic predictions in island biogeography (Wallace 1880).

Chapter 5 (published as Ó Marcaigh *et al.* 2022b) considered the “oceanic” islands of Menui and the Wakatobi archipelago, using monarch populations to test the supertramp hypothesis. Like the Sulawesi Babbler, the Pale-blue Monarch is endemic to Sulawesi and some of its offshore islands. Unlike the Sulawesi Babbler, it does occur on more distant islands like Taliabu (Eaton *et al.* 2021), which were not connected to Sulawesi by land bridges (Rheindt *et al.* 2020). This might indicate that the Pale-blue Monarch’s dispersal abilities are stronger than those of the Sulawesi Babbler. The Island Monarch does not occur on any islands occupied by the Pale-blue Monarch: many biogeographers take such exclusive island occupancy patterns as evidence for interspecific competition (Mayr and Diamond 2001), though there is debate about the robustness of this inference (Simberloff 1978). However, this chapter does not find evidence for a link between such competitive exclusion and the dispersal abilities of the birds, as might have been expected from the “supertramp strategy” (Diamond 1974). For one thing, the Pale-blue Monarch appears to maintain gene flow between all of the land-bridge islands, including Kabaena and Wawonii, such that these islands have continuous populations with Southeast Sulawesi (it is thus more dispersive than the Sulawesi Babbler or Red-backed Thrush). We found that the Island Monarch has diverged on a relatively small geographic scale, but did not find evidence that this species experienced selection against dispersal ability on relatively larger islands, such as Wangi-Wangi. Therefore, monarchs do not necessarily evolve to be extremely dispersive on small islands nor extremely sedentary on large ones, and their different dispersal abilities might be better explained as ecological responses to the taxon cycle rather than permanent evolutionary adaptations. Overall, Chapter 5 has provided another entry in an ongoing examination of the supertramp strategy (Diamond 1974, Mayr and Diamond 2001), and integration of this idea with the taxon cycle hypothesis (Wilson 1959, 1961, Ricklefs and Bermingham 1999, 2002). In this respect the chapter follows several other recent genetic studies on other supertramp taxa (Linck *et al.* 2016, Pepke *et al.* 2019).

The Olive-backed Sunbird is currently thought to occur over an extremely wide range, from India to Australia, with Sulawesi right at the centre. The availability of samples from across this range allowed Chapter 6 (currently under review at the Zoological Journal of the Linnean Society) to take in a wider geographic scope and consider the impacts of the biogeographic barriers that flank the Wallacea region. The Black Sunbird provided a contrast, overlapping with the Olive-backed Sunbird in the central part of its range. The work outlined in Chapter 6 found evidence that both of these taxa are composed of multiple species,

reflecting the influence of both small islands and large biogeographic barriers. Small islands provide distinct evolutionary environments and are famous for producing evolutionary divergence (Leroy *et al.* 2021), especially islands at the periphery of a species' range (Mayr 1947, Mayr and Diamond 2001). This chapter found a divergent Black Sunbird population on New Ireland in the Bismarck Islands, on the eastern periphery of its range. After the Olive-backed Sunbird is divided in three (Eaton *et al.* 2021), the Wakatobi Islands and Busuanga in the Philippines become "peripheral" islands as well, and these too hold divergent populations. The "Wakatobi Sunbird" exhibits signs of reduced dispersal on the small islands where it lives, when compared to the "Sahul Sunbird" on the much larger Sulawesi and land-bridge islands. Studies on supertramps have pointed out that conditions on larger islands should select against dispersiveness (Linck *et al.* 2016), but chapter 5 found no evidence that this had occurred in the Island Monarch. It must be noted that "large" is a relative term here, as a supertramp only inhabits small islands by definition. The sunbirds, on the other hand, live on landmasses as large as Australia and New Guinea, as well as very small ones. It seems this greater contrast in island size allows a relationship between island size and dispersiveness to take effect, although this relationship is in the opposite direction than predicted by the supertramp hypothesis.

Chapter 6 provides evidence that both Wallace's Line and Lydekker's Line (Figure 8.1) have had a greater influence on the evolution of sunbirds than is reflected in current taxonomy. This evidence also helps our understanding of these biogeographic ideas, as they have been treated in a "tangled" way in the literature (Ali and Heaney 2021). The division between sunbird species corresponds to the original and best-known version of "Wallace's Line" (Wallace 1863), rather than Huxley's (1868) version (drawn west of the Philippines), or Wallace's (1910) later revision (drawn east of Sulawesi). When first introduced, the term "Wallacea" included the Philippines as well as the central Indonesian islands (Merrill 1924, Dickerson *et al.* 1928). Later biogeographers excluded the Philippines (Darlington 1957). The patterns found in Chapter 6 support this later, "reduced" version of Wallacea, which has come to be more commonly used in the literature (Ali and Heaney 2021). The "Garden Sunbird" of the greater Philippines and the *aurora* birds in the western Philippines are evidently quite distinct from the "Sahul Sunbird" in Wallacea. It seems the Philippines ought to be treated separately from Wallacea by biogeographers, as the Philippine avifauna is richer in endemism than was previously appreciated (Lohman *et al.* 2010, Hosner *et al.* 2018).



**Figure 8.1.** Map of biogeographic boundaries considered in the text. The area hatched in blue represents the original conception of Wallacea (Merrill 1924), the area hatched in red the "reduced" version (Darlington 1957).

All of the previous chapters reinforce the idea that the speciation of birds on islands is an interplay between the isolating effects of water barriers and the dispersal ability of birds that enable them to cross between islands. Chapter 7 used a systematic review and a large Bayesian model to conduct a broad test of these ideas. The preliminary results were that birds with a low Hand-Wing Index or an understory lifestyle exhibited larger genetic distances between islands. This pattern was suggested by the differences in population structure between babblers (chapter 4) and more dispersive birds, like monarchs (chapter 5) and sunbirds (chapter 6). Chapter 7 extended this idea to a much wider cohort of birds and found that this general pattern holds true. The dataset assembled for Chapter 7 will be analysed further and will continue to cast light on speciation at a broad scale.

## 8.4 Conclusions and recommendations

This thesis has achieved its aims of helping to address some of the Linnean and Wallacean shortfalls in our knowledge of island birds, exploring the population structure of birds around Sulawesi and surrounding regions, and using the data to refine biogeographic theory around dispersal and speciation. Together, these chapters reveal how the permanent water barriers between oceanic islands impede gene flow and lead to genetic divergence, whether these barriers are as wide as Wallace's Line and Lydekker's Line (Chapter 6), or as narrow as the water barriers within Wallacea and Melanesia (Chapter 5). The shallow, short-lived water barriers around continental islands do not promote endemism to the same extent, but they can still produce distinct populations under certain circumstances (Chapters 3 and 4). Any intraspecific divergence could contribute to future speciation. The long-term isolation of continental fragments like Madagascar and Sulawesi has produced spectacular levels of endemism, which we are still working to catalogue and understand (Chapter 2). The extent to which inter-island water barriers drive genetic divergence is influenced by both the geological setting, making some barriers harder to cross, and by ecological and morphological traits, making some species less proficient at crossing a given barrier (Chapter 7).

These questions are becoming more important than ever as time goes on. This year marks thirty years since Raven and Wilson (1992) proposed a "fifty-year plan" to survey the world's biodiversity, in the publication that coined the term "Linnean shortfall". Most of that time has now elapsed, and yet the task

remains impeded by a lack of funding and support for the taxonomic work that is required (Engel *et al.* 2021). In the present economic landscape I share the anxiety of Wallace (1863), expressed at the beginning of this thesis, that the “pursuit of wealth” will drive much of Earth’s biodiversity extinct while it remains “uncared for and unknown”. The danger is apparent on the islands around Sulawesi, where industries like nickel mining threaten ecosystems and local communities alike (Morse 2019b, a).

These looming threats add urgency to the continued surveying, sampling, and sequencing of island birds. This thesis has highlighted certain islands and populations which should be prioritised in this effort. Around Sulawesi, populations that warrant further attention include the Sulawesi Babbler population of Wawonii, and the Island Monarch population on Menui and the islands to its north, as these might represent endemic subspecies. Taxonomic efforts would also be aided by the sampling of Island Monarchs and Black Sunbirds around Maluku, to clarify whether populations on either side of Lydekker’s Line are separate species. Lastly, the Bismarck Islands and the western Philippines hold genetically divergent Black Sunbirds and Olive-backed Sunbirds, respectively, which should be examined as potential cryptic species.

Large-scale datasets and modelling approaches have provided new ways to uncover the broad, general patterns of evolution, as seen in Chapter 7. However, the application of such methods can only tell us so much when basic information is lacking, especially regarding the number of species, their distributions, and their natural history. One sobering example is that the species distribution maps available to modellers are clearly faulty, as many of the supposed species boundaries are actually political and administrative borders (Hughes *et al.* 2021). Applied knowledge depends on basic knowledge, and so theoretical modelling approaches will be greatly improved as we gather more descriptive, foundational information on biodiversity.

Sequencing and modelling are only part of the solution: I echo the sentiment of Engel *et al.* (2021) that “new species are not found in the computers and sequencers of the big cities of the ‘North’, but in the natural and anthropized habitats of the entire planet and, especially, in the more biodiverse ‘South’”. The poverty of the “South” is thus an obstacle to the study and conservation of biodiversity (Reboredo Segovia *et al.* 2020), with the burning of Madagascar’s forests to produce cooking charcoal a particularly poignant example (Chapter 2). Such a condition has persisted for some time: Raven and Wilson (1992) saw the poverty of the developing world as an obstacle to their fifty-year plan. In an interview that was to contain some of his

last public statements, Wallace (1913) railed against the “horrible, grinding poverty” he saw afflicting many people, having become increasingly preoccupied with social issues. He died later that year.

Biologists from both the “North” and the “South” are putting in a valiant effort to surmount these many challenges. New initiatives are adding greatly to our knowledge of birds’ genomics (Feng *et al.* 2020) and traits, including data on morphology, ecology, and distributions (Tobias *et al.* 2022). Hopefully these new insights will help us to understand and conserve the “avian archipelago” while there is still an opportunity to do so. Island birds can inform us of geological history, the state of conservation, and evolutionary trajectories, or in other words “what is past, or passing, or to come” (Yeats 1928). I conclude this thesis with the hope that they will continue to do so, in Sulawesi, Madagascar, and around the world.

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