Evolutionary consequences of island colonisation in the silvereye: a genomic approach



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I declare that this thesis is entirely my own work, and except where otherwise stated, describes my own research. This work has not been submitted for any degree or professional qualification except as specified.

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Evolutionary consequences of island colonisation in the silvereye: a genomic approach

Abstract

Deterministic evolutionary outcomes are widespread across the Tree of Life. For example, many insular organisms show repeated evolution of some traits, many of which are captured under the so-called 'island syndrome'. A classic example is the repeated evolution of body size, but there are also less-explored consequences of island colonisation: a reduction in dispersal propensity, which is of particular interest because it can catalyse diversification. A great example of this concerns 'great speciators', bird species present on multiple islands that, at the same time, show many subspecies. Within the taxon cycle framework, great speciators are species that, after an initial range expansion stage, have differentiated into subspecies. However, this pattern is not entirely intuitive, since great speciators are hypothesised to have high dispersal propensity in the colonisation stage but low in the differentiation stage. This evolutionary puzzle is known as the "paradox of the great speciators". Strong selection against dispersal ability after island colonisation is often invoked to resolve this apparent paradox, but the mechanisms underlying it are not well understood. In Chapter 2, I explored the great speciator continuum and the drivers of incipient speciation on islands and found that spatial effects are particularly important in determining the occurrence of great speciators. The silvereye (*Zosterops lateralis*), of the white-eye bird family Zosteropidae, is a prolific natural coloniser of Southwest Pacific islands and has rapidly given rise to over 17 subspecies most of which are island-dwelling, making it a useful model to understand the evolutionary consequences of island colonisation. In Chapter 3, I used genome-wide data to build phylogenetic trees and networks to understand the evolutionary history of the silvereye. I found that continental subspecies are highly connected and can overcome major biogeographic barriers, while island populations are generally more differentiated. Island populations follow the island syndrome expectations: reduced dispersal propensity and increased body size. In Chapter 4, I took a candidate gene approach to explore whether the "paradox of the great speciators" can be understood from a genetic perspective in this species. I found that some behavioural genes, like *CREB1*, are associated with a reduction in dispersal, and that, in a partial migrant population, migrants carry longer *CLOCK* alleles than sedentary individuals. Chapter 5 explored the mechanisms underlying the repeated evolution towards a larger body size in island silvereyes and found that standing genetic variation is the most common route towards gigantism but that *de novo* mutations can also play a role. As a whole, this body of work advances our understanding of the mechanisms leading to repeated evolution after island colonisation and the impact on diversification.

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Publications and Author list

The following chapters consist of manuscripts that have been accepted for publication, published as preprints and in review, or in preparation. All chapters are a collaborative effort, although in each case the majority of work is my own.

CHAPTER 2. **Estandía, A.**, Merino Recalde, N., & Clegg, S.M. (2023). The great speciator continuum: incipient speciation on islands. *In preparation*

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CHAPTER 4. **Estandía, A.**, Sendell-Price, A. T., Oatley, G., Robertson, F., Potvin, D., Massaro, M., Robertson, B. C., & Clegg, S. M. (2023). Candidate gene polymorphisms are linked to dispersive and migratory behaviour: Searching for a mechanism behind the "paradox of the great speciators". ***Journal of Evolutionary Biology***, 36(10), 1503-1516. https://doi.org/10.1111/jeb.14222

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NMR: Conceptualization, Methodology, Software, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization. AE: Investigation, Data Curation, Writing - Review & Editing. SK: Writing – Review & Editing. EC: Supervision, Project Administration. BCS: Supervision, Project Administration, Writing – Review & Editing, Funding Acquisition.

Merino Recalde, N., **Estandía, A.**, Pichot, L., Vansse, A., Cole, E. & Sheldon, B. C. (2023). A densely sampled and richly annotated acoustic dataset from a wild bird population. ***bioRxiv*** https://doi.org/10.1101/2023.07.03.547484 | ***Animal Behavior*** (Accepted)

AE, LP, AV and NMR collected the data. NMR created the software and pipeline to plan fieldwork and analyse the data, annotated the dataset with help from AE, built the website, documentation, and visualisations, and wrote the original draft. BCS and EFC provided feedback and supervision throughout the research, and all authors contributed critically to drafts.

1

Introduction

Islands have inspired major ideas in evolutionary biology since the days of Charles Darwin and Alfred Russel Wallace. But what is so special about them? They are small and have discrete boundaries harbouring simplified communities and serving as natural laboratories to more easily study evolutionary processes in the wild (Emerson, 2002). While islands are home to endemic species and evolutionary radiations (Miles et al., 2023), they also limit the outcomes of adaptive processes, often leading to repeated evolution (Losos & Ricklefs, 2009). Some traits, like dispersal, show repeated evolution after island colonisation and can catalyse diversification (Waters et al., 2020). Understanding the complex interplay between a heterogeneous world, individual and species-level dispersal propensities, and the wide diversification rates seen across the Tree of Life can help us understand the emergence of insular diversity across space and time.

1.1 The role of geography and dispersal in divergence

> Species differences in dispersal are one of the most important explanatory factors underlying species differences in tendency to speciate, in geographic variability (level of endemism), and in breadth of distribution.
>
> *Mayr & Diamond, 2001, p. 66*

The diversity and distribution of species across space are largely determined by the interplay between geographical heterogeneity and the wide distribution of dispersal abilities across the Tree of Life. Geography can reduce opportunities for gene flow when populations become geographically separated, either by physical barriers or through colonising isolated habitats such as islands. Over time, the combination of distinct selective pressures and genetic drift can drive genetic differentiation, eventually leading to the formation of new species (Fitzpatrick et al., 2009; Mayr, 1942; Skeels & Cardillo, 2019). In geographically fragmented landscapes, the importance of dispersal becomes particularly pronounced as individuals move within and among habitats to maximise fitness, transferring alleles that reduce local adaptation and divergence (Barton, 2001; Ronce, 2007). Dispersal is a complex multivariate trait influenced by morphological, physiological and behavioural adaptations (Bonte et al., 2012). As such, evolutionary changes in dispersal ability are influenced by the genetic architecture, heritability and variance of the underlying traits (Saastamoinen et al., 2018). The genetic component of dispersal propensity can be determined by one or a few genes (e.g. the *pgi* locus has a direct effect on dispersal rate in the Glanville fritillary butterfly, *Melitaea cinxia*; (Haag et al., 2005; Niitepõld & Saastamoinen, 2017), but is most often of polygenic origin. Polygenic traits tend to show higher standing variation and have higher evolutionary potential and evolvability, increasing the chances of rapid adaptive evolution in a new environment (Yeaman, 2022). Whatever its genetic basis, a moderate dispersal propensity can increase the chances of repeated colonisation of new habitats, like islands (Alzate & Onstein, 2022; Yamaguchi, 2022). Naturally, flying organisms have an advantage over terrestrial animals: they can actively fly across water, which has made them the focus of research in diversification on islands (Grant, 2001; Miles et al., 2023).

1.2 Evolution on islands

Islands are hotspots of biodiversity (de Bruyn et al., 2014; McCullough et al., 2022) and observations of island populations have shaped major ideas in evolutionary biology and ecology, including the evolution of populations through natural selection, best exemplified perhaps by the famous adaptive radiation of Darwin's finches in the Galápagos (Grant & Grant, 2002). The diversity of species on islands is a product of complex interactions between biotic and abiotic factors. Two abiotic factors, island size and proximity to the mainland, significantly influence biodiversity (Losos & Ricklefs, 2009; MacArthur & Wilson, 1963). Larger islands tend to harbour more species, while more isolated islands generally have lower species richness due to limited opportunities for species to colonise. However, it is important to note that biotic interactions also play a critical role in shaping island biodiversity. For example, some insular species depend on the presence of others for their survival, such as certain terrestrial birds and the plants they rely on (Ciarle & Burns, 2023).

1.2.1 Repeated evolution: the island syndrome

Islands provide environments where comparable evolutionary pressures can result in the rapid emergence of similar phenotypes (Baeckens & Van Damme, 2020). The 'island syndrome' refers to the phenomenon where sets of morphological, physiological, and behavioural traits evolve in a predictable way when organisms colonise islands. The syndrome ultimately results from the exposure to a common suite of biotic conditions on islands: a combination of reduced predation and parasitism, and a shift in the balance of intra- and interspecific competition towards the former. These factors fundamentally change the selective pressures in predictable and repeatable ways (Clegg & Owens, 2002; Lomolino, 2005; Meiri et al., 2004). Arguably the best-characterised component of this syndrome is the 'island rule' that describes evolution towards medium body sizes in insular settings; small colonising species evolve larger body sizes and vice versa (Benítez-López et al., 2021). Additionally, island-dwelling species commonly display reduced dispersal capacity, characterised by i) 'behavioural flightlessness', a reluctance to cross gaps of water (Diamond 1981); and ii) morphological changes, like the loss of wings in beetles (Waters

et al., 2020) or a tendency to adopt a more ground-dwelling lifestyle in birds (Wright et al., 2016). Island species also tend to exhibit a slower pace of life, typically producing fewer offspring while providing higher levels of parental care (Jezierski et al., 2023).

1.2.2 Diversification on islands: the taxon cycle

Island colonisation often leads to population differentiation, and ultimately to the emergence of new species (Whittaker et al., 2023). The taxon cycle theory offers a simple overview of the speciation process through range expansions and contractions (Wilson, 1961), which Ricklefs & Cox 1972 divided into four stages (Fig. 1.1):

- Stage I: The cycle begins when a dispersive species colonises multiple islands. During this stage, the species is commonly referred to as a "supertramp" (Diamond, 1974). Examples of supertramps include some cuckooshrikes (Pepke et al., 2019), white-eyes (Linck et al., 2016; O'Connell et al., 2019), honeyeaters (Mapel et al., 2021) and diving beetles (Balke et al., 2009; Toussaint et al., 2013).
- Stage II: These isolated populations start to diverge due to limited gene flow between them. Over time, this can lead to the development of distinct subspecies or incipient species. Populations on small islands tend to become extinct due to small population sizes, creating gaps in the distribution of the species.
- Stage III: The populations that are able to persist colonise the highlands, outcompeting other taxa and freeing the coastal habitat for new colonisers.
- Stage IV: Once populations have substantially differentiated, taxa form part of relatively stable communities, especially on large and mountainous islands. In this stage, species are high-altitude specialists and have small range sizes.

This theory was first proposed to understand ant diversity in Melanesia (Wilson, 1961) and, since then, multiple studies have found evidence to support it in a variety of taxa and archipelagos (lizards, Losos et al., (1992); ants, Matos-Maraví et al., (2018); birds, Jønsson et al., (2014), Pedersern et al., (2018); humans, Keegan et al., (1987)).

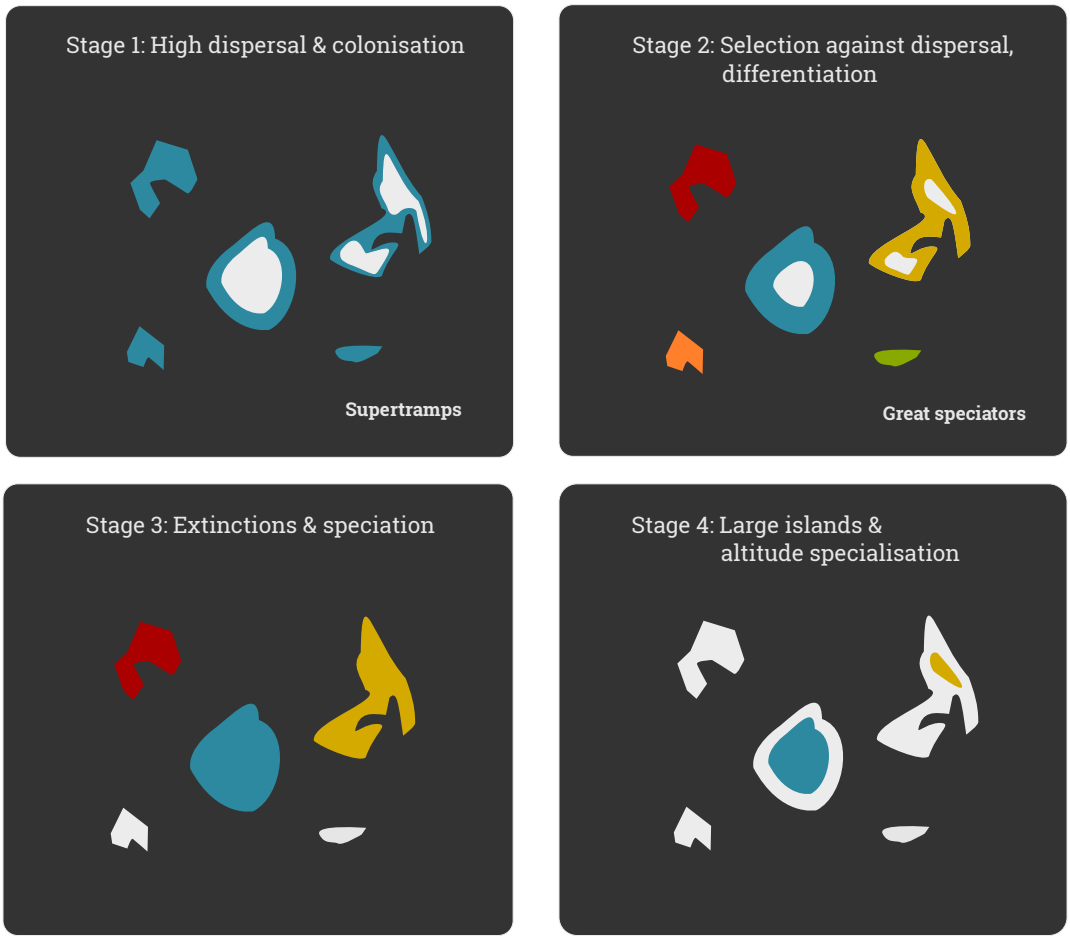


Figure 1.1: The taxon cycle theory proposes that species on islands evolve through range expansions and contractions.

1.2.3 The "paradox of the great speciators"

The "paradox of the great speciators" was first proposed after an expedition to the island-rich region of Northern Melanesia (Diamond et al., 1976). Over two decades later, Mayr and Diamond (2001) compiled a list of 31 Northern Melanesian 'great speciators'. Northern Melanesia encompasses the Bismarck and Solomon archipelagos, more than 90 volcanic islands of highly variable size that harbour an extraordinary diversity; from human languages (Rumsey, 2019) and genetic diversity (Friedlaender et al., 2008; McCullough et al., 2022) to phenotypically distinct populations of birds (Andersen et al., 2015; Jønsson et al., 2010). Diamond et al. (1976) proposed

that the combination of persistent colonisation attempts and a generalist ecology increased the chances of survival and colonisation success, while their morphological differentiation was due to rapid adaptation to local conditions. However, if dispersal ability remains high, gene flow among sister populations or with the source population is predicted to slow divergence (Mayr, 1963). To explain the observed distribution and diversity, they invoked strong selection acting against dispersal on each of the newly established island populations. This directional selection may be driven by the energetic cost of this behaviour (McNab, 2002) or the risks of overwater dispersal, which are often higher than any possible demographic pay-off (Bonte et al., 2012). The popular hypothesis of a post-colonisation reduction in dispersal ability has mostly been proposed as a post-hoc explanation of observed patterns (Andersen et al., 2015; Manthey et al., 2020; O'Connell et al., 2019; Weeks & Claramunt, 2014). A few studies have attempted to formally test for a shift in dispersal ability, mainly using morphological proxies, but they show conflicting results, with some supporting changes in dispersal while others do not (Kennedy et al., 2016; Pedersen et al., 2018). The incongruence in the results is not surprising, as the shift from dispersive to sedentary lifestyle might initially involve behavioural flightlessness rather than morphological changes (Diamond, 1981).

1.2.4 Studying divergence on islands through a genomic lens

A genomic approach is fundamental to infer accurate population structure and gene flow patterns, reconstruct past demographic changes, and explore whether variation arises neutrally or has been the result of a selective process (Beichman et al., 2018). The increasing accessibility of next-generation sequencing allows scientists to obtain vast amounts of genomic data from diverse populations at a fraction of the cost and time compared to traditional sequencing methods, which has expanded our ability to study evolution in natural populations (Ekblom & Galindo, 2011; Ellegren, 2013). For example, we can now reconstruct the evolutionary history of rapidly radiating species and time-calibrate the colonisation of islands providing a solid baseline for future questions. We can also identify candidate genes and molecular pathways associated with key adaptive traits. For example, extensive research has been conducted on classic island study systems, such as Darwin's finches, but it is only recently that we have comprehensive genomic data

to explore the mechanistic basis of the evolutionary processes at play (Enbody et al., 2023).

1.3 Study system: The silvereye (*Zosterops lateralis*)

Understanding the emergence of great speciators and the repeated evolution of the phenotypes that make up the island syndrome requires a model system that permits temporal calibration of phenotypic and genomic changes, as interpretations can vary greatly depending on whether a group experienced a rapid burst of diversification after a dispersive phase or if diversification is caused by the accumulation of subspecies with time. The silvereye (*Zosterops lateralis*) is an ideal system to study the evolutionary consequences of island colonisation, because of its broad island distribution and high subspecific diversity resulting from independent waves of colonisation (Fig. 1.2). This species is part of the Zosteropidae family which counts with more than 120 species of white-eyes, yuhinas, and allies (Clements et al., 2021). This group of passerines is one of the highest-diversifying lineages within vertebrates (Moyle et al., 2009) and many members have been classified as great speciators (Mayr & Diamond, 2001). The silvereye is represented by 17 morphological subspecies distributed across the Australian mainland, Tasmania, New Zealand, New Caledonia, Vanuatu, Fiji, French Polynesia, and other smaller islands in the Southwest Pacific (Fig. 1.3) (Mees, 1969). Some of the island populations represent ancient colonisations where we can find distinct subspecies, such as those in Southern Melanesia, Lord Howe Island, and Heron Island. However, other populations are very recent natural colonisations for which we have historical data of arrivals, like the stepping-stone colonisation of the two islands of Aotearoa New Zealand, Chatham Islands, and Norfolk Island (Mees, 1969). Additionally, we know of a human-introduced population on the island of Tahiti in French Polynesia, from which individuals have moved to other islands and keep spreading across the archipelago to this day (Thibault & Cibois, 2017). The silvereye island colonisation success is attributed to excellent dispersal ability, and colonisation by flocks followed by rapid population expansions (Sendell-Price et al., 2021), which together reduce the severity of founder effects (Estoup & Clegg, 2003); while their rapid morphological differentiation in novel environments is likely a product of their initial generalist diet and short-generation time (Scott et al., 2003).



Figure 1.2: A Capricorn silvereye (*Z. l. chlorocephalus*) on Heron Island, Australia. Photograph by Andrea Estandía in April 2022.

The silvereye complex offers a remarkably diverse system characterised by independent colonisations and a variety of divergence timeframes. Together with the availability of a scaffold-level reference genome for the Lifou subspecies *Z. l. melanops* (Cornetti et al., 2015), the silvereye makes an ideal species for investigating the evolutionary consequences of island colonisation.

1.4 Aims of thesis

In this thesis, I explore the mechanisms underlying the evolutionary consequences of island colonisation in a great speciator: the silvereye.

In **Chapter 2**, I explore whether great speciators represent the end of a continuum, if they share a common evolutionary history, and what characteristics they might have in common. To do this, I create an extensive global database of bird subspecies distribution on islands and calculate

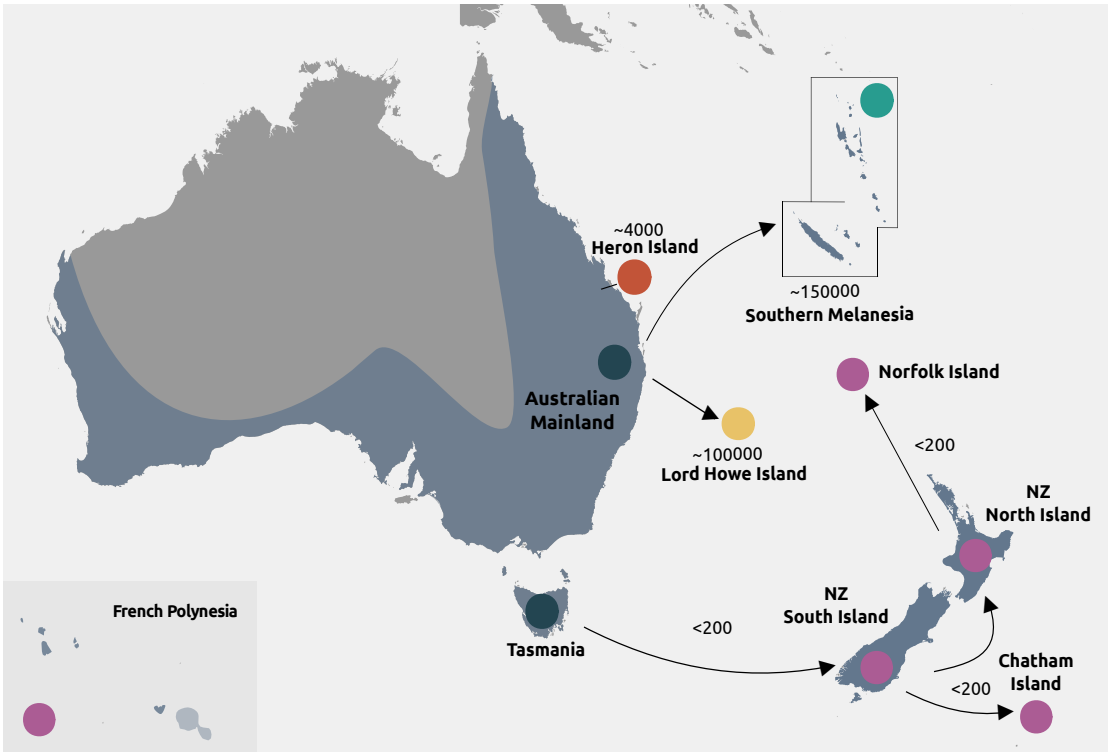


Figure 1.3: Map showing the silvereye distribution highlighted in blue and the time of colonisation of each island or archipelago. French Polynesia is shown as inset.

a continuous 'great speciator index' for each species, along with a range of spatial, environmental, and morphological variables. Using Bayesian generalised linear phylogenetic models I find that great speciators lie at the high end of a continuous distribution and that spatial effects seem to be particularly influential underscoring the importance of the geographical context in the emergence of great speciators.

In **Chapter 3**, I explore the evolutionary history of the silvereye by building phylogenetic trees and networks with genome-wide data from more than 100 individuals of silvereye. I resolve key evolutionary relationships but find phylogenetic conflicts at shallow nodes, potentially a product of rapid and recent radiation, and ongoing gene flow. I estimate that the silvereye lineage diverged from other South Pacific *Zosterops* species approximately 1.5 million years ago and rapidly after, two main silvereye clades emerged: Southern Melanesia and the broader South Pacific (encom-

passing Australia, New Zealand, and outlying islands). This study resolves the main evolutionary relationships of the silvereye and emphasises the idea that islands generally lead to population differentiation, a key aspect in the emergence of great speciators.

In **Chapter 4**, I explore whether selection against dispersal ability explains the high number of subspecies seen in insular silvereyes. To do this, I generate data for six candidate genes linked to migration and animal personality differences in 20 South Pacific populations of silvereye that range from highly sedentary to partially migratory. I detect genetic associations in three of the six genes: i) in a partial migrant population, migrant individuals have longer alleles at the *CLOCK* gene compared to resident individuals from the same population; ii) *CREB1* display longer average allele lengths in recently colonised island populations (< 200 years), compared to evolutionarily older populations. Bayesian broken stick regression models support a reduction in *CREB1* length with time since colonisation and decreasing dispersal propensity. This pattern differs from neutral genetic structure, suggesting potential selection against dispersal after colonisation; and iii) like *CREB1*, *DRD4* shows differences in polymorphisms between recent and old colonisations. These results indicate the "paradox of the great speciators" can be partially understood through a genetic lens.

Organisms can rely on new advantageous mutations, but these arise slowly. A more common route towards the evolution of similar phenotypes in recently diverged lineages is selection on existing variation. In **Chapter 5**, I sequenced whole genomes in the silvereye, a small bird that follows the "island rule", which involves the repeated evolution of increased body size after island colonisation. Both mechanisms are evident in this system: a new putative mutation is linked to body size in one colonisation event, while standing genetic variation seems crucial in all colonisations. These findings emphasise that even at shallow divergences, new mutations can contribute to parallel evolution.

Finally, in the general discussion (**Chapter 6**), I discuss the main results of Chapters 2-5 and future directions of research.

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2

The great speciator continuum: incipient speciation on islands

In Preparation

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The great speciator continuum: incipient speciation on islands

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The speciation process on islands is complex, influenced by the interplay between dispersal, geographic heterogeneity, and other species traits. 'Great speciators' are bird species present on many islands that, at the same time, have many subspecies. This pattern is considered paradoxical because their relatively wide distribution suggests good dispersal abilities but population differentiation often requires low levels of gene flow and therefore limited dispersal. This evolutionary puzzle was highlighted by Mayr and Diamond in the 1970s, based on field observations in Northern Melanesia. Since their first publication, the 'great speciator' concept has been used extensively but not consistently: it has been applied to different taxonomic levels and is often confused with diversification rates. Here we expand this concept to allow a quantitative, global analysis. By compiling a global database of island bird subspecies and developing a 'great speciator index', we identify some key factors influencing the extent of early diversification within island bird species. We find that great speciators are the end of a continuum rather than a discrete group and that this is an evolutionarily labile property. Spatial effects seem to be particularly influential in driving the 'great speciator' pattern, more so than habitat heterogeneity or species traits, which have weaker but predictable effects. Our preliminary study advances the understanding of great speciators by introducing a quantitative approach, challenging categorical distinctions, and revealing the diverse nature of factors contributing to early population divergence in island bird species, highlighting the need for further exploration of scale-dependent patterns and species-specific colonisation history.

islands | diversification | birds | biogeography | great speciator | range size | dispersal

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Introduction

Some species are more prone to differentiate than others, and different traits may promote divergence along the speciation continuum (Jablonski 2008). Among these, dispersal is arguably one of the most important. Dispersal abilities vary greatly across the Tree of Life, which, in interaction with geographical heterogeneity, contributes to shaping species diversity across space. This is because the new populations established by highly dispersive species should remain well connected, which, by allowing gene flow, limits divergence (Diamond 1977; Slatkin 1987; Garant et al. 2007). Interestingly, some species that are present across many islands also have high levels of morphological diversity within them (Diamond et al. 1976), which would seem to lead to a contradiction: to reach many different islands a species needs to be a good coloniser, while having differentiated populations suggests limited dispersal and reduced gene flow (Diamond et al. 1976). This pattern of population differentiation is seen in various island taxa, including beetles (Papadopoulou et al. 2009; Suárez et al. 2022), bats (Speer et al. 2017), and birds (Spurgin et al. 2014; Estandía et al. 2023). In the island-rich region of Northern Melanesia, located in the South Pacific, this evolutionary puzzle is historically known as the 'paradox of the great speciators', a term coined by Jared Diamond and Ernst Mayr in the 1970s to refer to some particularly prolific subspeciators. The 'original' great speciators included representatives of several taxonomic groups, with raptors, rails and passerines among them. Most of these species were abundant, short-distance colonisers with many subspecies, the latter often endemic to single islands (Mayr and Diamond 2001). The 'great speciator' term soon took a life of its own, having been co-opted to refer to various taxonomic levels—species, genus, and family—since the original publication, as well as clades with fast diversification rates (Moyle et al. 2009).

The popularity of the term suggests that it might be a useful construct, as it identifies species with characteristics of interest to researchers interested in the origins of diversity and the drivers of cladogenesis across the Tree of Life. However, currently, great speciators are treated as a discrete group based on arbitrary thresholds, and this makes it difficult to study this phenomenon quantitatively and at a global scale. Whether they merely represent the end of a continuum is an empirical question, as is if they share a common evolutionary history. Here, we first try to answer these questions, and then explore the impact of various hypothesised factors—spatial, environmental, and morphological—on the variation in the number of subspecies across islands. Finally, we discuss whether giving rise to high subspecific richness on islands will translate into diversity at higher taxonomic scales.

To do this, we create an extensive global database of bird subspecies distribution on islands and calculate a continuous 'great speciator index' for each species, along with a range of spatial, habitat, and morphological variables. We aim to identify the key factors influencing diversification in island birds, contributing to a deeper understanding of both the enduring evolutionary puzzle surrounding great speciators and the broader origins of biodiversity.

Results and discussion

Great speciators are the end of a continuum.

Mayr and Diamond (2001) proposed a set of fixed rules used to determine whether a Northern Melanesian bird was a great speciator (see Methods: *Identifying a Great Speciator*). These rules were useful to represent the concept they tried to convey but they cannot be easily scaled to perform a global analysis. To explore whether this categorical concept represents one end of a continuum of different levels of differen-

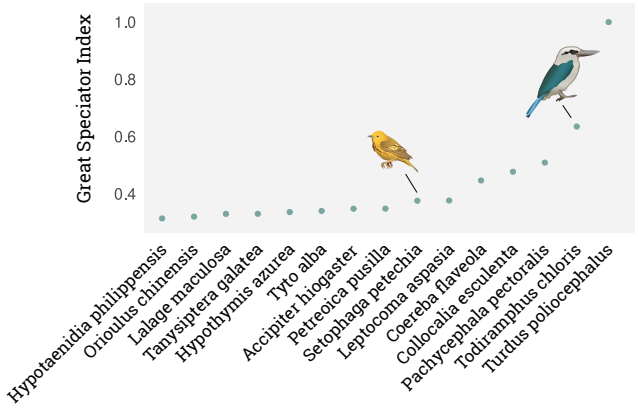


Fig. 1. Top 15 species ranked by the Great Speciator Index. The illustrations represent two species found at the high end: the Collared Kingfisher (*Todiramphus chloris* and the Yellow Warbler (*Setophaga petechia*)

tiation on islands across spatial scales, we introduce a 'great speciator index' (GSI). This GSI shows a continuous distribution, with species usually referred to as 'great speciators' found at the very end of its frequency distribution (Fig. 1; Table 1). Many of these species were mentioned in the original list for Northern Melanesia, including the island thrush *Turdus poliocephalus* and the collared kingfisher *Todiramphus chloris*, while others were absent primarily due to taxonomic updates resulting from more recent molecular studies (Pedersen et al. 2018; Jønsson et al. 2019; O'Connell et al. 2019; McCullough et al. 2022). Birds from other regions also scored high—this is not an exclusively Northern Melanesian phenomenon—including the bananaquit *Coereba flaveola*, or the yellow warbler *Setophaga petechia*. These are small passerines that live in Central and South America and are present in most of the Antilles, where they have more than 25 subspecies, most of which are island endemics. Some globally widespread species, which would not fit the original definition of a great speciator, are also at the higher end of the distribution. These include the barn owl *Tyto alba* and the great tit *Parus major*, due to their high number of subspecies on islands that are well separated by whole oceans or continents, in what is likely a simpler case of isolation by distance. For example, the barn owl has subspecies in the Antilles, Galápagos, the Canary and the Solomon Islands, but rarely more than two within each archipelago. We suspect that the mechanisms leading to a high GSI can be many and varied, but all of the species at the end of the continuum share an unusual diversity and are interesting in their own right. Some might help us to understand how species achieve broad distributions in a general sense, like the quasi-circumglobal barn owl, while others might be more relevant when it comes to exploring the drivers of divergence on smaller island chains.

Being a great speciator does not strongly depend on evolutionary history.

If being a great speciator depended on characteristics of the species that either evolve slowly or are otherwise not very evolutionarily labile, we would expect that phylogenetic relatedness would explain a large fraction of the total variation in our GSI. In contrast, the GSI exhibited a low to moderate phylogenetic signal (Fig. 2E; Phylogenetic intraclass correlation (ICC): n = 2762; Estimate=0.23; 95% CI [0.06,0.51])). With the inclusion of other predictors in the model, the adjusted estimate of the phylogenetic signal decreased further (Fig. 2E; Estimate=0.1; 95% CI [0.01,0.31]). This suggests that some of the variables included in the analysis are themselves phylogenetically conserved—which is indeed the case for, e.g., body mass and hand-wing index—and covary with GSI, thus absorbing part of the variance explained by phylogeny alone. That the phylogenetic signal is weak indicates that a great speciator pattern may arise from similar environmental and geographical characteristics or other, evolutionary labile traits that are similar due to convergent evolution rather than a common evolutionary history. Indeed, we find species with high GSI from different orders, including Columbiformes, Passeriformes, Apodiformes, Gruiformes and Coraciiformes. This finding aligns with the lack of phylogenetic signal observed in avian subspecies richness globally (Sol et al. 2005; Phillimore et al. 2007).

Spatial and biogeographic factors are associated with the probability of being a great speciator.

Once we adjust for phylogenetic relatedness, spatial effects seem to be particularly influential in driving the 'great speciator' pattern, underscoring the importance of geographical context. We found that the maximum length of a species' range and the distance between the islands that it occupies have a stronger effect than variables capturing habitat heterogeneity or morphology, although with much uncertainty around effect size estimates (Fig. 2B). Species that have extensive ranges, adjusting for the separation between islands within them, are less likely to be great speciators (-0.66, CI 95% [-1.21, -0.09]). At the same time, a species is more likely to be a great speciator if the islands where it is present are well separated (0.63, CI 95% [0.15, 1.10]). When a species with a relatively small range (which indicates low dispersal ability) inhabits a very fragmented landscape, gene flow is expected to be low, leading to a higher probability for population differentiation (Kisel and Barraclough 2010; Ashby et al. 2020; Broyles et al. 2023). However, smaller ranges can be explained without invoking the active colonisation of multiple islands. Instead, island populations can be achieved through other mechanisms such as vicariance if the ancestor had a broader distribution achieved through land bridges and sea-level rises during the Pleistocene resulted in a fragmented landscape. This hypothesis seems to fit some patterns of intraspecific differentiation within island archipelagos (Leonard et al. 2015; Cros et al. 2020).

Beyond simple spatial properties of the territories they occupy, species are present in different biogeographic realms, which vary geographically, climatically and ecologically from one another (Holt et al. 2013), driving variability in the occurrence of great speciators. Even after adjusting for any potential residual effect of the number of islands not already captured by the GSI itself, Oceania has a posterior predicted mean GSI across all species that is higher than most other realms (0.032; CI 95% [0.017, 0.053], Fig. 2A). Ocea-

Table 1. Top 15 species ranked by the Great Speciator Index

Common Name	Scientific Name	Number of Islands	Number of Subspecies
Island Thrush	*Turdus poliocephalus*	82	46
Collared Kingfisher	*Todiramphus chloris*	763	44
Golden Whistler	*Pachycephala pectoralis*	194	28
Glossy Swiftlet	*Collocalia esculenta*	504	31
Bananaquit	*Coereba flaveola*	213	25
Purple-rumped Sunbird	*Leptocoma aspasia*	209	21
Yellow Warbler	*Setophaga petechia*	1256	28
Pacific Robin	*Petroica pusilla*	47	14
Variable Goshawk	*Accipiter hiogaster*	246	20
Barn Owl	*Tyto alba*	1505	26
Black-naped Monarch	*Hypothymis azurea*	299	20
Common Paradise Kingfisher	*Tanysiptera galatea*	58	14
Polynesian Triller	*Lalage maculosa*	104	16
Black-headed Oriole	*Oriolus chinensis*	396	20
Buff-banded Rail	*Hypotaenidia philippensis*	446	20

nia is an ideal region for species to diverge: it is exclusively composed of islands, most of which are volcanic and have a wide variety of ages (Neall and Trewick 2008). This is a dynamic region where islands rapidly appear and disappear, subject to sea level changes and cataclysmic events, and climatic phenomena including the frequent El Niño can facilitate the colonisation of Pacific islands by multiple organisms, including humans (Goodwin et al. 2014). On the other side of the spectrum are the Nearctic and Palearctic, where the average predicted GSI are lower relative to other realms (0.011, CI 95% [0.007, 0.016], and 0.013, CI 95% [0.009,0.018], respectively, Fig. 2A), agreeing with previous results showing that the North hemisphere harbours the least island endemic richness (Kier et al. 2009).

Does habitat heterogeneity promote the emergence of great speciators?.

Habitat heterogeneity is expected to promote divergence and species richness (Phillimore et al. 2007; Udy et al. 2021). Regions closer to the equator, for example, have high habitat and resource heterogeneity in a relatively stable climate (Fine 2015). These conditions promote greater trophic specialisation, allowing incipient specialist species to persist over time (Fine 2015; Saupe et al. 2019). In contrast, at high latitudes, lineages are often more ephemeral because resources are more susceptible to climatic changes, and if they vanish, so do specialists (Futuyma 2010). Supporting this idea, we found a negative latitudinal gradient in the GSI (-0.15, CI 95% [-0.25, -0.05]; Fig. 2C), where species with high GSI are more common at low latitudes, independently of other features that covary with latitude, such as range size. Precipitation range, which should be a more direct proxy for habitat heterogeneity (Tuanmu and Jetz 2015; Rodríguez et al. 2019), showed a weak positive effect on the GSI once we adjusted for latitude, but not temperature range (Fig. 2C).

Another factor contributing to habitat heterogeneity and the availability of diverse ecological niches is elevation. The median elevation of the islands occupied by a species had a positive effect on the GSI (0.1, CI 95% [0.03, 0.16]; Fig. 2C), which may indicate that species that are found across a wider elevation range might be more likely to give rise to locally adapted forms, thus increasing subspecies richness. Note that our data does not allow us to determine if these subspecies are currently situated in the highlands or the lowlands; it simply indicates a tendency for the occupied islands to have higher elevations on average. Additionally, this association may be influenced by a known positive relationship between island size and elevation (Hoffman et al. 2019). Larger islands offer diverse habitats, more opportunities for divergence, and host a greater number of species and subspecies: this covariation between elevation, area and habitat heterogeneity complicates drawing definitive conclusions.

Weak effects of dispersal ability and body mass.

Although weak, there is a negative association between the Hand-Wing Index (HWI), which is a proxy for dispersal ability (Sheard et al. 2020; Weeks et al. 2022), and GSI (-0.08, CI 95% [-0.01, -0.16]; Fig. 2D), suggesting that species with higher GSI are less likely to be long-distance dispersers, as proposed by Diamond et al. (1976). While birds with low dispersal ability may reach distant islands through alternative means, such as storms or changes in sea level, higher-GSI species tend to have smaller ranges, well-separated islands, and lower HWI. All these factors point towards lower dispersal capacity being a crucial factor, as previously suggested by Mayr and Diamond (2001) and Diamond et al. (1976).

Additionally, smaller birds tend to have higher GSI (-0.1, CI 95% [-0.18,-0.02]; Fig. 2D). This can be explained by a combination of characteristics: First, smaller birds tend to flock together, resulting in a larger number of colonisers (Clegg et al. 2002) which can help to prevent population extinction due to inbreeding, environmental stochasticity, or predation. Second, small birds more often have a generalist ecology, which increases their opportunities to find an available niche (Scott et al. 2003). Finally, their short generation times enable populations to rapidly recover from founder effects and promote

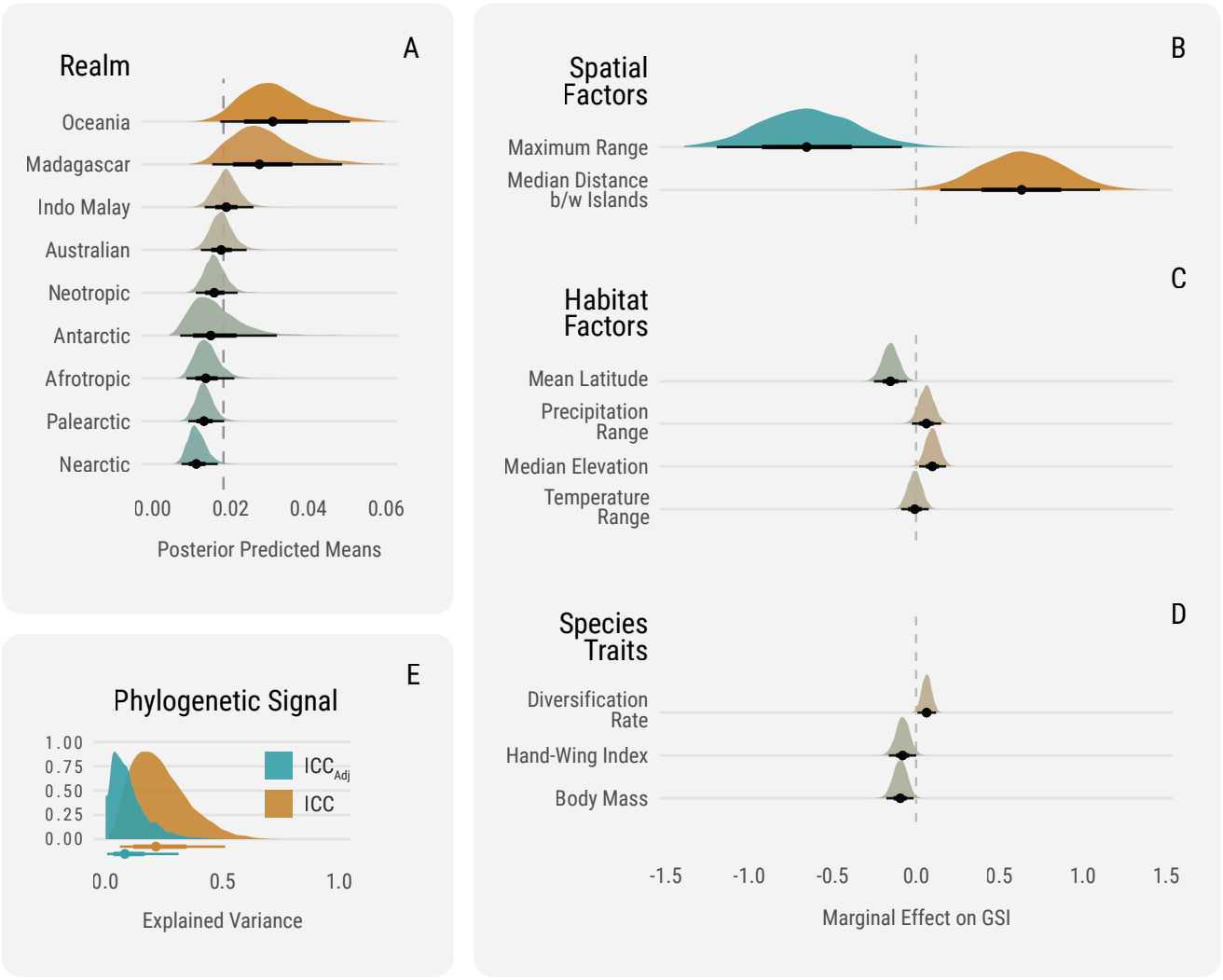


Fig. 2. Model estimates of the marginal effects of different variables on the Great Speciator Index.
Panel A shows the posterior predicted means for each biogeographic realm, arranged by mean. The discontinuous line represents the overall mean GSI across all regions. Panels B, C, and D show the posterior distributions of marginal effects at the mean for each variable in the model, with dots and whiskers representing the median estimate and its 95% credible intervals. Panel E shows posterior estimates of the proportion of the total variance explained by the phylogeny, with and without the rest of the model covariates.

rapid morphological evolution (Sendell-Price et al. 2020).

Species with higher GSI have higher diversification rates.

The term 'great speciator' has been applied to species or groups of organisms that have undergone rapid and extensive diversification, resulting in the emergence of multiple distinct species. For instance, the genus *Zosterops* is often referred to as a great speciator, even though many of its member species lack subspecies (Clements et al. 2021). The term's polysemy likely stems from its original use coexisting with a second that is strongly implied by the fact that having species is a property of higher taxonomic levels (e.g., a genus or a family with many species rather than a species with many subspecies).

While the processes determining diversity at different taxonomic levels may well be similar, the temporal scale and magnitude of changes differ greatly. Subspeciation involves differentiation within a species, resulting in divergent populations that show some trait differences. However, these differences may not persist long enough to give rise to distinct species, making subspecies more ephemeral, as differentiation can be easily reversed through gene flow (Dobzhansky 1958). In contrast, fully-fledged speciation involves more substantial changes, often accompanied by the development of reproductive barriers that prevent gene flow between populations. Although some level of introgression may occur across closely related species (Schluter and Rieseberg 2022), the changes associated with speciation are typically enduring. It is important to note that these are just different phases in the speciation process.

According to our analysis, species with higher diversification rates are more likely to be great speciators. Although this effect is among the weakest in our analysis, it supports the long-held assumption that by producing more lineages, some of them will persist and fully become distinct species, agree-

ing with the idea that species and subspecies diversification rates are correlated in birds (Phillimore 2010). However, it is important to note that the species included here vary in age, which means that variation in the number of subspecies used to calculate the GSI may be biased by changes in subspeciation probability with time.

Conclusions.

In this study, we aimed to unravel the nature of 'great speciators' by investigating whether they represent a distinct category or are part of a continuum of differentiation in island bird populations. Our results indicate that the concept of great speciators is better understood as the end of a continuum, with a continuous distribution captured by the introduced GSI. This index demonstrated that species spanning different taxonomic orders and regions exhibit high subspecific richness on islands. Our analysis revealed a weak phylogenetic signal in the GSI, suggesting that being a great speciator is not strongly dependent on evolutionary history. Instead, our findings highlight the influence of spatial and biogeographic factors in driving the great speciator pattern. Specifically, the maximum length of a species' range and the separation between islands within that range emerged as crucial factors. Regions with a dynamic island landscape showed a higher mean GSI, emphasising the importance of geographical context in fostering divergence.

Habitat heterogeneity, especially at lower latitudes, was weakly associated with the emergence of great speciators, supporting the idea that diverse ecological niches contribute to speciation. Elevation also played a role, suggesting that species occupying islands with a broader elevation range are more likely to give rise to locally adapted forms. Dispersal ability, represented by the Hand-Wing Index, exhibited a weak negative association with the GSI, suggesting that great speciators are less likely to be long-distance dispersers even after adjusting for range size. Finally, our analysis indicated that species with higher diversification rates are more likely to be great speciators, aligning with the idea that the processes leading to subspecies and species diversification are interconnected. However, it is crucial to consider the temporal dynamics of speciation phases when interpreting these results, since different mechanisms could lead to similar patterns.

Understanding the relationship between spatial factors, which our analysis shows as having the largest effects, and GSI will require more in-depth analysis of scale-dependent patterns, such as the interaction between range size, arrangement of islands, and dispersal ability, which are only indirectly represented in our analysis. A preliminary model of the interaction between range size and distance between islands shows that maximum GSI is achieved when ranges are small but islands are maximally distant within them, which agrees with the idea that great speciators are short-distance colonisers.

Our dataset includes species of varying ages, some having had more time to give rise to differentiated populations, others the product of recent radiations. The analysis amalgamates all species, providing estimates of average patterns across birds. However, understanding the mechanistic basis for any correlations will require detailed knowledge of each species and subspecies' age and colonisation history. At the same time, analysing taxonomic levels below the species is challenging: phenotypic differences are often not clear-cut at the early stages of divergence, leading to taxonomic hypotheses that might not represent monophyletic lineages or an underestimation of subspecific diversity. In 2006, only 57% of island avian subspecies were phylogenetically distinct (Phillimore and Owens 2006). However, with the increasing use of molecular phylogenies for taxonomic classification, this number is likely substantially higher now. We argue that while this poses challenges for specific species, general patterns will likely hold unless subspecific richness suffers from systematic biases that are correlated with our predictor variables.

The 'great speciators' have been a focus of interest across evolutionary biologists since the 20th century. However, it was unclear whether great speciators had common characteristics that contributed to their diversity. Here, we aimed to examine the great speciator phenomenon quantitatively and, in this way, contribute to our understanding of the complex dynamics underlying incipient speciation on islands. In summary, our study advances the understanding of great speciators by introducing a quantitative approach, challenging categorical distinctions, and revealing the multifaceted nature of factors contributing to early population divergence in island bird species.

Methods

Species Distribution Data.

We collected resident and breeding distribution data for 10079 bird species in shapefile format from BirdLife International and the current taxonomy for 11,758 bird species and 20,062 subspecies from the BirdLife Taxonomic Checklist v5. Using vectorised maps downloaded from Natural Earth (naturalearthdata.com) we created a landmass shapefile where each closed polygon was considered an independent landmass, retaining only those that had a smaller area than Madagascar. This size threshold was chosen as the great speciator concept is an inherently allopatric one, and sympatric bird speciation is thought to be very rare on islands smaller than Madagascar (Coyne and Price 2000). Nevertheless, intra-island divergence resulting in subspecies (whether geographically allopatric within the island or not) does occur (e.g. the common tailorbird (Orthotomus sutorius) has two subspecies coexisting in Sri Lanka; del Hoyo et al. 1992), and those cases were removed from the analysis. We intersected each of the 10079 species distribution shapefiles with the landmass shapefile. Species that were not distributed on the landmass shapefiles were discarded (i.e. those with distributions exclusively continental or on islands the size of Madagascar and above), leaving 6,523 species and 14,816 subspecies. We counted the number of islands where each species was present.

Some species were represented by subspecies on both islands and continental/large islands. We counted only those

subspecies present on islands using descriptions of the subspecies ranges in the Clements Checklist v2021 (Clements et al. 2021). We chose the Clements Checklist over the Birdlife International checklist as the latter does not contain subspecies distribution information and subspecies distribution ranges were not available in shapefile format. In the 7904 cases where subspecies ranges were not available in the Clements Checklist, we manually compiled distribution information for each subspecies from descriptions in Avibase (http://avibase.bsc-eoc.org/). In a new column, we manually assigned 1 to the subspecies that were present on an island smaller than Madagascar or 0 to those that were not and counted the number of island subspecies per species. For example, out of the 16 subspecies of silvereye (Zosterops lateralis), 5 are mainland species while 11 live on islands. We assigned a 1 to each of those 11 island subspecies and a 0 to mainland subspecies. This protocol produced the number of islands occupied by each species and the number of subspecies per species across those islands (5525 subspecies on islands).

Identifying a Great Speciator Index.

Mayr and Diamond (2001) applied a set of rules to classify great speciators: i) if it has seven or more subspecies across its range; or ii) if it has four to six subspecies and if the ratio between the number of subspecies and the number of islands where the species is present is higher than 0.33. This categorical classification is accepted for identifying great speciators at the regional scale of Northern Melanesia, however, the set of rules is not generalisable at larger spatial scales. This is because subspecies number increases as range increases but not in a linear fashion (Phillimore et al. 2007), introducing a similar issue to the scaling pattern of occupancy (Kunin 1998), when identifying great speciators on regional versus global scales.

To develop a quantitative index, our initial idea was to use the residuals of regressing the number of subspecies on the number of islands (Approach 1). This represents how much each species deviates from the mean population-level trend in the expected number of subspecies given the number of islands where they are present. However, although conceptually easier to interpret, these residual values are distributed in a way such that they are very difficult to model. As an alternative, we developed a 'Great Speciator Index' (GSI), consisting of the number of subspecies divided by the logarithm of the sum of the number of landmasses where the species is distributed, or GSI = Nsubspecies / log(Nislands), scaled to lie between 0 and 1 for ease of interpretation, with higher values indicating the highest ranked great speciators. By logging the denominator only, we effectively retain information on the number of subspecies while accounting, at least in part, for the scaling issues of the number of islands (Fig. S1; Kunin 1998; Xiao et al. 2011). The extracted residuals from approach 1 and the GSI were highly correlated (Pearson correlation coefficient = 0.92), so we decided to use the latter as the response variable as the GSI proved to be easier to model. We realise that the interpretation of this index is not as straightforward but still captures the fundamental idea of a great speciator.

Ecological variables.

We collated information on seven biogeographical and abiotic variables that may influence biodiversity patterns, in this case, patterns of subspeciation. Opportunity for subspeciation may be higher for species with greater range size, more geographically isolated populations on average, and greater climate heterogeneity across that range, or it may vary across biogeographical realms of the world simply because of the arrangement of landmasses. We collected information on annual temperature range, annual precipitation range and median elevation for each species obtained from WorldClim v.1 (with a resolution of 2.5 minutes of a degree). Ranges were calculated as the subtraction of the maximum value minus the minimum value. We calculated range size as the distance between the two most distant polygons within the distribution of a species. To capture the geographic distribution of the occupied islands by each species, we created a distance matrix among all islands where a species is present and computed the median distance between islands.

To determine if there were differences among the world's regions in the production of great speciators, for each species in our database we collected information on the absolute latitude values (measured as the mean latitude of each species distribution) and biogeographic realm occupied (Sheard et al. 2020).

Finally, we compiled the following morphological variables for each species: Hand-wing Index (a widely used proxy for dispersal ability; Claramunt et al. 2011) and body mass from Pigot et al. (2018).

Diversification rates.

We estimated the diversification rate for each species in our dataset, to determine if great speciators tend to have high diversification rates. We used BAMM (Bayesian Analysis of Macroevolutionary Mixtures) to calculate species-specific rates of diversification or tip-specific diversification rates on the Hackett topology (Hackett et al. 2008). We computed the maximum clade credibility tree including only those species with available genetic data (6663 species). To control for incomplete taxon sampling, we calculated the percentage of species per family with genetic information, using the Jetz et al. (2012) taxonomy, and including this variable in the BAMM analysis, an approach used in multiple studies (Rabosky et al. 2013).

We analysed this topology performing four independent chains and 200 million iterations of reversible jump Markov chain Monte Carlo (rjMCMC) discarding 10% as burn-in. We set a mean of 0.02 on the rate parameter of the Poisson distribution as it is recommended for trees containing more than 5000 tips. Default priors were applied for the other rate parameters. To check for convergence, we calculated the effective sample size (ESS).

Biogeographical and ecological correlates of great speciator propensity.

We fit Bayesian phylogenetic generalised linear mixed models in brms (Bürkner 2017), a library to fit Bayesian models

using stan (Stan Development Team 2020). We tested the effect of the biogeographic and ecological variables on the GSI: hand-wing index, body mass, diversification rate, realm, absolute latitude, range size, median distance to all other occupied islands, temperature range, precipitation range, and median elevation. We log-transformed body mass, range size, and median distance to all other islands. All quantitative variables were scaled from 0 to 1 to improve interpretability. To control for phylogenetic relatedness, we included a phylogenetic variance-covariance matrix (G matrix) calculated using the Jetz et al. (2012) phylogeny, as a random variable. We modelled the response distribution as a truncated log-normal with a hurdle (logistic) part to account for the zeroes (produced by species that have only one subspecies in a single island). We set weak regularising priors for all parameters on the z-score-normalised scale, with a normal distribution centred on 0 and a standard deviation of 2.
We ran the model with four independent chains of 1500 iterations, discarding 300 as warm-up. To check for convergence, we inspected the trace plots and checked that the ESS > 1000, which is sufficient for stable estimates (Bürkner 2017). We performed prior and posterior predictive checks to ensure that our priors were reasonable and that we could recover relevant distributional properties of the outcome variable.
First, to calculate the strength of the phylogenetic signal (the tendency of related species to resemble each other more in the response variable than species drawn at random from the same tree), we ran a bare-bones model to calculate the variance in GSI explained by the phylogeny alone. The phylogenetic signal (Intraclass Correlation Coefficient for, ICC, often referred to as lambda is computed using the formula

$$\frac{V_P}{V_P + V_R}$$

where

$$V_P$$

represents the variance explained by phylogeny, and

$$V_R$$

is the residual variance of the model. Since certain traits that have a high phylogenetic signal (e.g., body size and HWI) are also correlated with the GSI and might contribute to the phylogenetic signal found in the latter, we also recalculated the now adjusted residual variance explained by the phylogenetic variance-covariance matrix in the full model (Fig. 2E).

Author contributions

AE, NMR and SMC formulated the idea and SMC supervised the project. AE curated the database and performed all analyses with input from NMR. AE drafted the manuscript with input from NMR.

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Data and code availability

Code to reproduce all analyses is available at https://github.com/andreaestandia/1.0_great_speciators

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3

Islands promote diversification within the silvereye clade: a phylogenomic analysis of a great speciator

In Preparation

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Islands promote diversification within the silvereye clade: a phylogenomic analysis of a great speciator

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Geographic isolation plays a pivotal role in speciation by restricting gene flow between populations through distance or physical barriers. However, the speciation process is complex, influenced by the interplay between dispersal ability and geographic isolation, especially in "great speciators" – bird species present on multiple islands that, at the same time, have many subspecies. Comparing population differentiation in both continental and insular settings can help us to understand the importance of geographical context in the emergence of great speciators. The highly diverse white-eye family Zosteropidae includes several great speciators, including the silvereye (*Zosterops lateralis*) which consists of 16 subspecies, 11 occurring on islands. The distribution of the silvereye on the Australian continent and numerous southwest Pacific islands allows us to explore the influence of different forms of geographic isolation on population divergence. To do this, we conducted a comprehensive phylogenomic analysis of the silvereye and compared patterns of population divergence in insular versus continental silvereye populations. We estimate that the silvereye lineage emerged approximately 1.5 million years ago, followed by the split of the two main silvereye clades: Southern Melanesia and the broader South Pacific (encompassing Australia, New Zealand, and outlying islands). Continental populations show low genetic population structure, which suggests that they can overcome multiple forms of geographic barriers across long distances. In contrast, most island populations are highly structured even over relatively short distances. Divergence statistics further support the idea that water barriers lead to a higher population differentiation when compared to continental distances. Our results indicate that islands promote divergence and provide an empirical example of the geographical conditions that result in the emergence of great speciators.

phylogeny | *Zosterops* | great speciator | dispersal | silvereye | radiation | islands

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Introduction

Geography plays a key role in the speciation process by limiting allele exchange in distant and isolated populations, resulting in divergence (Anderson & Weir, 2022; Bolnick & Fitzpatrick, 2007; Coyne & Allen Orr, 1998). Spatially separated populations may experience isolation-by-distance whereby individuals that are close to each other are likely to be more related than those farther apart (Wright, 1943). Additionally, geographical barriers, such as mountain ranges, deserts or water barriers, can further promote divergence (White, 2016). Oceanic islands and archipelagos are hotspots of biodiversity and home to evolutionary radiations (Miles et al., 2023), thanks in part to their isolation, which acts as a geographical barrier that limits gene flow (Emerson, 2002). Highly dispersive species can colonise distant islands and, by virtue of this vagility, continue to connect populations through gene flow resulting in little population structure (Andersen et al., 2015; Bohonak, 1999; Suárez et al., 2022). However, some taxa, the so-called 'great speciators', represent a seemingly paradoxical case. These bird species are present on multiple islands, which indicates high dispersal ability, while having many subspecies, which requires reduced gene flow (Diamond et al., 1976).

Great speciators can be thought of as species with multiple differentiated populations sampled at a particular point along the speciation continuum. Under the 'taxon cycle' framework—the idea that speciation on islands proceeds through phases of range expansion and contraction (Wilson, 1961)—a great speciator has undergone the first two phases of a cycle: an initial expansion followed by a reduction in dispersive behaviour that promotes population differentiation and subspeciation. This pattern can be seen in multiple south-west Pacific birds, including kingfishers (Andersen et al., 2018; DeRaad et al., 2023; O'Connell et al., 2019), corvids (Pepke et al., 2019) and passerines (Estandía, Sendell-Price, Oatley, et al., 2023; Klicka et al., 2023; Pedersen et al., 2018).

Water barriers can be more effective at driving population differentiation compared to continental distances (Broyles et al., 2023; Estandía & Merino Recalde, 2024). Comparing divergence patterns between continental and insular settings can help us to understand the importance of the geographical context in the emergence of great speciators. A fundamental first step in doing this is to have a good understanding of their evolutionary history. Traditionally, phenotypic traits were used to reconstruct phylogenetic relationships, but island taxa often show repeated morphological evolution (Benítez-López et al., 2021), which might lead to an incorrect topology. Genomics now allows us to reconstruct evolutionary relationships with increased confidence (Lee & Palci, 2015), although it is still challenging for those clades that radiate rapidly, such as many great speciators (Moyle et al., 2009). The condensed sequence of cladogenetic events and increased levels of incomplete lineage sorting (ILS) driven by rapid radiation (DeRaad et al., 2023) and recent divergence (Irestedt et al., 2013) complicate phylogenetic inference (Meleshko et al., 2021). Additionally, gene flow can lead to reticulate evolution (Xu, 2000). Conflictive nodes can be identified by using a combination of multiple phylo-

genetic approaches (Smith et al., 2020) and dense sampling across the genome can provide reliable phylogenetic reconstructions if sources of error are appropriately handled (Kapli et al., 2020).

The white-eye family, Zosteropidae, which originated in southeast Asia (Gwee et al., 2020), has undergone a rapid expansion into the Palaeotropics and Oceania within the last 2 million years (Moyle et al., 2009), giving rise to over 120 species (Clements Checklist v2021). In their studies of northern Melanesian avifauna, Mayr & Diamond (2001) identified *Zosterops griseotinctus* as a great speciator based on the number and distribution of its morphological subspecies. *Zosterops* species outside of this region can also be categorised as great speciators, including the southwestern Pacific silvereye (*Zosterops lateralis*). This species has at least 16 morphological subspecies (11 on islands and the rest on the Australian continent) (Mees, 1969). The island populations have been hypothesised to represent multiple independent colonisations from the Australian mainland (Mees, 1969), with the most recent colonisation from Tasmania to New Zealand and outlying islands being historically documented (Clegg et al., 2002; Sendell-Price et al., 2021).

On the Australian mainland, five subspecies of silvereye are currently recognised based on morphology and plumage patterns (Mees, 1969; Schodde & Mason, 1999). Banding records show they can disperse long distances (Fig. S1) and two subspecies display partial migration: members of the Tasmanian subspecies (*Z. l. lateralis*) and a southern mainland subspecies present in Victoria and New South Wales (*Z. l. westernensis*) journey up to 1,500 km north in the winter. There is evidence of substantial gene flow among eastern Australian subspecies (Estandía, Sendell-Price, Oatley, et al., 2023), but relationships with other mainland subspecies are unclear. Those from Western Australia (*Z. l. chloronotus*) and South Australia (*Z. l. pinarochrous*) are separated from each other and eastern Australian subspecies by major biogeographical barriers. These barriers include the Nullarbor Plain, a desert that stretches approximately 1000 kilometres between Western and South Australia, and the Eyrean barrier, a historical biogeographical barrier inferred to have been present in South Australia. Although the Nullarbor Plain covers a vast area, it has had a lesser impact on current avian diversity compared to the Eyrean barrier (Dolman & Joseph, 2015).

Islands might promote diversification in silvereye populations relative to their continental counterparts. To explore the role of geography in driving divergence in this great speciator, we sequenced the whole genomes of 114 individuals from 8 different white-eye species, including 12 silvereye subspecies from islands and the Australian continent. We explored the phylogenomic relationships among the silvereye complex and related white-eye species to i) reconstruct the evolutionary history of the silvereye; which then provides the basis to ii) compare connectivity patterns within continental versus island subspecies; and iii) determine the role of geography in promoting population differentiation and incipient speciation.

Methods

Field sampling.

We sampled seven South Pacific white-eye species (the silvereye *Z. lateralis*, representing 12 subspecies, the Vanuatu white-eye *Z. flavifrons*, the Louisiade white-eye *Z. griseotinctus*, the large Lifou white-eye *Z. inornatus*, the small Lifou white-eye *Z. minutus,* the slender-billed white-eye *Z. tenuirostris*, and the green-backed white-eye *Z. xanthochroa*) and the Réunion grey white-eye *Z. borbonicus*, which served as an outgroup (Table S1). Birds were caught in the wild using mist nets or hand-operated traps. A blood sample of 20–40 µl was collected from the brachial wing vein and stored in 1 ml of Queen's lysis buffer (Seutin et al., 1991). We took a suite of morphological measurements: wing length (maximum flattened chord of the longest primary feather measured with a metal ruler), tail length (the length of the central tail feathers from the base to the tip measured with dividers); metatarsal length, head length from the rear of the skull to the bill tip, and bill length, width, and depth at the posterior nostril opening, all measured with dial callipers.

DNA extraction, library preparation, and sequencing.

We extracted DNA from blood samples using a standard phenol-chloroform protocol (Seutin et al., 1991). We added 100 µl of blood sample to a microcentrifuge tube containing 250 µl of DIGSOL extraction buffer (0.02 M EDTA, 0.05 M Tris-HCl (pH 8.0), 0.4 M NaCl, 0.5% sodium dodecyl sulphate (SDS)) and 10 µl of Proteinase K (20mg/mL). Samples were incubated at 55°C overnight. Following incubation, we added 250 µl of phenol:chloroform:isoamyl alcohol (25:24:1) and gently mixed the samples for 10 minutes, then centrifuged at 10,000 rpm for 10 minutes. We transferred the aqueous layer to a new microcentrifuge tube and repeated the previous step by adding the phenol mixture, mixing and centrifuging it. We recovered the aqueous phase, transferred it to a new tube, added 250 µl of chloroform:isolamyl alcohol (24:1), and mixed and centrifuged the samples as in the previous steps. We precipitated the DNA by adding 2 volumes of ice-cold 100% ethanol, 1 volume of 2.5M ammonium acetate, and 2 µl of glycogen, leaving it overnight at -20°C, then centrifuging the tubes for 10 min at 15,000 rpm at 4°C before removing the supernatant. We rinsed the precipitate with 500 µl of ice-cold 70% ethanol and centrifuged the tubes as in the previous step. We removed the supernatant and left the precipitated DNA to dry at room temperature. Once dried, the DNA was resuspended in 50 µl of TE (Tris-EDTA) buffer (0.01 M Tris-HCL (pH 8.0), 0.0001 M EDTA). We quantified the DNA concentration with a Qubit 2.0. DNA extracts were sent to Novogene UK for library preparation and whole genome sequencing at 5X on the Illumina Novaseq 6000 platform (Illumina, San Diego) using paired-end 150bp sequencing reads.

Quality control and genotype calling.

We trimmed reads to remove adapter content and base calls of low quality using fastp (Chen et al., 2018) with default settings and 10 bp trimmed from the start of each read.

We aligned cleaned reads of each individual to a pseudochromosome assembly for this species (Estandía, Sendell-Price, Robertson, et al., 2023) using the Burrows-Wheeler Aligner (BWA)-mem algorithm (Li & Durbin, 2010). We calculated genotype probabilities in ANGSD (Korneliussen et al., 2014) using the samtools model and keeping SNPs with a minimum allele frequency of 0.05 and a mapping quality score 20. We filtered the bam files by excluding SNPs that showed significant deviation from Hardy-Weinberg equilibrium and excluded any loci putatively under selection with PCAngsd (Meisner & Albrechtsen, 2018) to have an unbiased neutral dataset that allows accurate population structure analyses (Kirk & Freeland, 2011). We further filtered our dataset by only retaining those sites that were placed on autosomes for which genotype probabilities were high (100% confidence), allowing us to call genotypes and obtain a VCF file with 21,726 SNPs suitable for analyses of population structure and phylogenetic reconstruction.

Phylogenetic reconstruction.
We conducted three independent maximum likelihood analyses with IQ-TREE (Nguyen et al., 2015) with the most appropriate model for our data, selected using ModelFinder (Kalyaanamoorthy et al., 2017). As SNPs-based datasets only contain variable sites and can lead to branch length overestimation (Leaché et al., 2015), we applied ascertainment bias correction. We obtained support values by running 100 bootstraps and applying a 25% burn-in.
Concatenated approaches typically do poorly in the presence of ILS (Warnow, 2015). An alternative approach, the multispecies coalescent model, offers a solution by inferring phylogenies while accounting for ancestral polymorphisms and gene tree-species tree conflict (Edwards et al., 2016). We estimated species trees using two coalescent-based methods: SNAPPER, implemented within BEAST2 (Bouckaert et al., 2014); and SVDquartets, implemented within PAUP* (Swofford & Sullivan, 2003) which is a method robust to the presence of gene flow (Long & Kubatko, 2018).
SVDquartets takes multi-locus single-site data, infers the quartet trees for all subsets of four species, and then combines these trees into a species tree (Chou et al., 2015). We used SVDquartets to build 100,000 random quartet trees with the main genomic dataset (21,726 SNPs). We then explored uncertainty in relationships by producing 100 bootstrap replicates. We used Figtree (Rambaut, 2009) to visualise a majority-rule consensus tree, whereby only relationships that appear in at least 50% of rival trees are resolved. Since SNAPPER is more computationally intensive, we randomly selected 1500 SNPs, where all loci were called at high confidence. We set four priors: 1. *Z. borbonicus* was set as an outgroup based on previous white-eye phylogenies (Gwee et al., 2020); 2. the silvereye was set as an independent clade; 3. we time-calibrated the root of the tree by applying a normal distribution with an offset at 2.25 Mya, mean of 0.8 and SD of 1, ensuring that 95% of the probability falls within the timeframe when *Zosterops* emerged (Leroy et al., 2021); and 4. we set a prior for the emergence of the Capricorn silvereye (*Z. l. chlorocephalus*) at 4,000 years old with a SD of 1,000 as the islands that the subspecies currently inhabits were formed approximately 4,400 years ago and have been vegetated for no more than 4000 years (Clegg et al., 2008; Hopley et al., 2007). We ran three independent analyses with a Markov Chain Monte Carlo (MCMC) chain of 100,000 steps with a burn-in of 10,000 steps. We visualised the full set of trees with Densitree (Bouckaert, 2010), highlighting inconsistencies when there were conflicting tree topologies.
To explore the occurrence of reticulate evolution we used Splitstree4 (Huson & Bryant, 2006) and built a Neighbor-Net, which visualises genetic distance between individual samples as a phylogenetic network (Bryant & Moulton, 2004). This approach helps represent complex evolutionary histories that do not fit neatly into a bifurcating tree structure.

Population structure.
We performed a genomic PCA with PCAngsd and decomposed the resulting covariance matrix into eigenvectors to explore population structure visually. We also conducted admixture analyses with NGSadmix (Skotte et al., 2013) by setting a maximum of 22 clusters, the total number of species plus silvereye populations sampled. To explore substructure within two highly divergent groups, we created two separate datasets (Dataset 1: Southern Melanesia (SM) and Dataset 2: Australia, New Zealand and Outlying Islands (ANZO)) and repeated the PCA and admixture analyses described above. We used WGSassign (DeSaix et al., 2023) to assign individuals to populations. When populations are genetically distinct, individuals can be assigned to their population of origin with high confidence, whereas highly admixed populations result in low confidence assignments and many individuals are 'misassigned' to non-origin populations. We defined populations at the level of island or continental region (State or Territory of Australia, except for the Australian Capital Territory, which was treated as part of New South Wales). We used a leave-one-out approach, removing one sample at a time from the sampled population, recalculating that population's allele frequency, and then estimating how likely it was for that sample to belong to each of the tested populations. Following DeSaix et al., (2024) we calculated posterior probabilities of assignment to a population by dividing the maximum likelihood of assignment over the sum of all likelihoods.

Gene flow.
In addition to ILS, admixture across highly divergent lineages could lead to phylogenetic conflict. We examined if admixture among mainland species explains unresolved nodes. We restricted this analysis to mainland subspecies because most widely used models assume constant population sizes and no selection (Smith & Hahn, 2023), an assumption that is violated in island silvereye populations that have experienced size fluctuations associated with colonisation and establishment (Estoup & Clegg, 2003). We explored patterns of population splits and mixtures in the history of mainland subspecies with Treemix (Pickrell & Pritchard, 2012), which fits a model based on population allele frequencies and a Gaussian approximation to genetic drift. We generated maximum

likelihood trees taking into account linkage disequilibrium by grouping 1000 SNPs into blocks. We tested a range of migration events (m from 0 to 15) and estimated the optimal migration edges with OptM (Fitak, 2021), which resulted in a maximum of four migration events.

Morphological distinctiveness.

We trained a Random Forest Classifier on a dataset of morphological data from 1292 individuals from 21 silvereye populations (Table S2) for which we had measured the following traits: wing, tail, metatarsal and head length, posterior bill length, and anterior bill width and depth. The model training process was divided into three steps:

- Data Resampling: To address the marked class imbalance, we resampled the data with replacement so that each population had the same number of samples. This was done by grouping the data by population and sampling 15 instances from each group. This process was repeated 100 times to get a better estimate of the variance in model performance on random subsets.
- Model Training: We split the resampled data into training and testing sets, trained a Random Forest Classifier on the training data, and made predictions on the testing data. At each iteration, we added the confusion matrix of the predictions to an average confusion matrix and stored the feature importances to quantify uncertainty derived from stochasticity in both model performance and data selection.
- Model Evaluation: Model Evaluation: After training we evaluated the model's performance on the testing data. This included calculating accuracy, generating a confusion matrix, and performing a permutation test. We generated several plots to visualise the model's performance, including a permutation test score plot (Fig. S2), a confusion matrix heatmap, a hierarchical clustering of the confusion matrix, and a feature importance plot (Fig. S3).

The goal of this process was to train a model to classify populations based on their morphology, on the assumption that the degree of misclassification between pairs of populations is indicative of their overall morphological (dis)similarity based on a non-linear combination of traits. This, in combination with the molecular data, can inform us of the extent to which genomic divergence is paralleled by morphological changes, and highlight cases of either parallel morphological changes or conservatism.

Testing the geographical drivers of population differentiation.

To explore whether water gaps promote differentiation more than continental distances, we built a model in *brms* (Bürkner, 2017) that incorporated: i) pairwise F_{ST} as the outcome variable, which represents population differentiation and was calculated with ANGSD; ii) the logarithm of distance between each of the populations as a predictor, iii) and whether the comparison was between islands or between continental populations, also as a predictor. We also tested for an interaction between the two predictors and added a multi-membership grouping term. We used MCMC with four chains of 4000 iterations each, including a warm-up of 400 iterations. We evaluated convergence via visual inspection of the MCMC trace plots, checking that the ESS>200 and the R values for each parameter (R=1 at convergence).

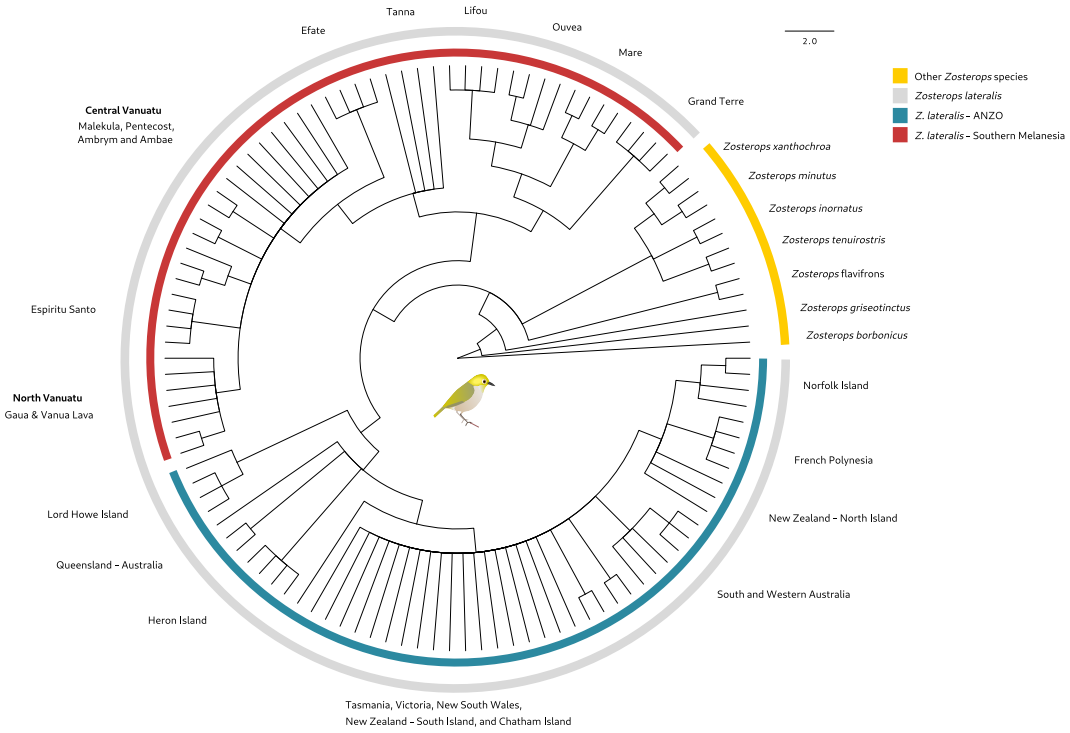
Results

Phylogenomic analyses.

We used several approaches to explore the evolutionary relationships of this group of birds: maximum-likelihood with IQ-TREE (Fig. S4), coalescence model-based SVDquartets with PAUP* (Fig. 1A), and Bayesian inference with SNAPPER (Fig. 1B). All analyses agreed on a split between South Pacific *Zosterops* species and *Z. borbonicus*, which we estimated to have occurred around 2.17 Ma (95% credibility intervals (CI)=1.45-2.9) (Fig. 1; Fig. S5). The Louisiade white-eye *Z. griseotinctus* was the earliest group to branch off, followed by *Z. flavifrons*, and then *Z. tenuirostris*. Among these, the split between *Z. tenuirostris* and the three other non-silvereye species in New Caledonia (*Z. inornatus*, *Z. minutus*, and *Z. xanthochroa*) showed different patterns of relatedness depending on the analytical method used: SNAPPER supported *Z. tenuirostris* as sister to the three species of New Caledonian white-eyes (Fig. 1B), IQ-TREE proposed placing it as the sister of the Large Lifou white-eye *Z. inornatus* (Fig. S4), and SVDquartets indicated a polytomy (Fig. 1A). All analyses showed that *Z. minutus* and *Z. xanthochroa* were more closely related than *Z. inornatus* and *Z. minutus* that occur as single-island endemics on Lifou. The silvereye split from other *Zosterops* species approximately 1.5 Ma (95% CI=0.98-2.01), rapidly forming two separate clades representing Southern Melanesia, which split into Vanuatu and New Caledonia, and ANZO (those from Australia, New Zealand, and the Outlying Islands), with Lord Howe Island as the basal lineage of this group. Despite showing high support for the overall divergence pattern at these nodes, the credibility intervals for their ages are wide (Fig. S5). While these deeper relationships were supported by high bootstrap values and posterior probabilities, shallower ones often showed less support. For example, Southern Melanesia showed complex relationships: SVDquartets indicated a split between New Caledonia and Vanuatu (Fig. 1A). However, SNAPPER suggested Tanna as the basal lineage, followed by Mare (Fig. 1B), while IQ-TREE suggested an early split of a clade containing Mare and Grand Terre (Fig. S4).

Another clade where low support was particularly noticeable was among mainland subspecies and recent colonisations in the ANZO group where some relationships were fully unresolved. Heron Island *Z. l. chlorocephalus* and Queensland *Z. l. cornwalli* individuals were either: part of the same clade (IQ-TREE), formed a polytomy (SVDquartets, Fig. S4), or Heron Island emerged as sister to all mainland subspecies and recent coloni sations (SNAPPER) (Fig. 1B), with a divergence age of approximately 80,000 years ago (95% CI=0.53-

A)



B)

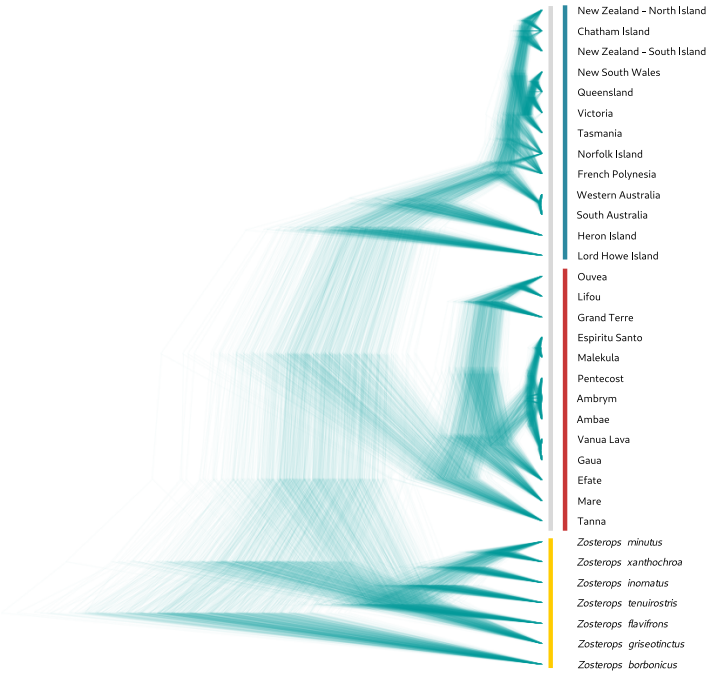


Fig. 1. Phylogenomic reconstruction of the silvereye clade and other South Pacific white-eyes. A) Phylogenetic relationships across South Pacific white-eyes, including a dense sampling of silvereye subspecies using a coalescent-based approach with SVDquartets. B) Variability in the inferred phylogenetic relationships with SNAPPER, highlighting areas where the tree space is more densely populated. Phylogenetic conflict occurs at shallow nodes including among Vanuatu islands, continental silvereyes, and recently colonised populations.

1.13) (Fig. S5). The mainland populations and recent colonisations showed differences depending on the method used but some patterns emerged repeatedly: South and Western Australia were a single cluster, the North Island of New Zealand, Norfolk Island and French Polynesia often grouped together, as well as Chatham Island and the South Island of New Zealand. Finally, silvereyes from Tasmania and the Eastern coast of Australia (Victoria and New South Wales) were part of the same clade.

Population structure: genomic and morphological data.

We visualised genetic population structure by plotting the two PCA axes that explained most genomic variation and the results from the admixture analysis. We repeated each analysis using three datasets: i) the entire sample set including all silvereyes and white-eye outgroups, ii) a subset only including silvereye samples from the ANZO group, and iii) a subset including only samples from Southern Melanesia. Together, these analyses supported the existence of three major groups: Southern Melanesian silvereyes, ANZO silvereyes and other *Zosterops* species. The first principal component in the PCA of the entire sample set explained 38.4% of the variation, and separated Southern Melanesian silvereyes from all other silvereye populations and other *Zosterops* species, while the second (20.9%) separated all silvereyes from other *Zosterops* species (Fig. 2A). Within Southern Melanesia, the silvereyes from the New Caledonian archipelago (Grand Terre and the Loyalty Islands) were distinct from those from North and Central Vanuatu islands, with Efate and Tanna populations intermediate between the two archipelagos (Fig. 2A). These groupings, along with the distinctiveness of Tanna and Efate, were supported by admixture plots (Fig. 2B). Assignment tests showed that individuals from Tanna, Efate, Gaua, Espiritu Santo, and Pentecost were correctly assigned to their original populations (Table S3). In contrast, individuals sampled from populations in the Central and Northern Vanuatu region were incorrectly assigned to Pentecost. Additionally, we observed a significant level of structure within the New Caledonian archipelago.

Within the ANZO group, the PCA revealed three distinct clusters: Lord Howe Island, Heron Island, and a larger cluster comprising Australian mainland samples, Tasmania, and recently colonised populations (Fig. 2A). This was also supported by the admixture analysis, with the historically documented colonisations showing some finer substructure (Fig. 2B) that was also evident in assignment tests. The main patterns to note were: Western Australia and South Australia were part of the same cluster; overlapping genetic profiles among widely separated eastern Australian sites (Queensland, New South Wales, and Victoria), and the continental island of Tasmania; and the recently colonised populations showed a similar genetic makeup, except for Norfolk Island and French Polynesia where individuals were mostly classified under their own cluster.

Training a Random Forest classifier on morphological data revealed that some island populations, such as those from Tanna, Lord Howe, and Grand Terre, could be accurately distinguished from other silvereye populations based on their morphology alone (Fig. S3). Other Southern Melanesian populations sometimes clustered together, especially across Central Vanuatu (Malekula, Espiritu Santo and Pentecost), and two of the Loyalty Islands (Lifou and Ouvea). Heron Island was clustered with islands from Vanuatu despite not being close genetically. This result is not surprising as island populations frequently experience parallel evolution of body size, a phenomenon known as the 'island rule'. In line with the genetic results, it was harder to morphologically distinguish among recently diverged populations and among populations sampled on the Australian mainland.

Network analyses, admixture and gene flow.

The Neighbor-Net agreed with other methods on the main split between other *Zosterops* species, Southern Melanesian silvereyes and the ANZO group (Fig. 3A). However, Lord Howe Island emerged as an independent lineage closer to other *Zosterops* species than to silvereyes. When we analysed the ANZO subset in detail, which included Lord Howe Island, we found that the samples from this island, Heron Island and Queensland showed mild reticulation at the base (Fig. 3C). Reticulation was more evident in samples from South and Western Australia, and Victoria and Tasmania, a pattern consistent with extensive gene flow across mainland populations, as supported by the Treemix results (Fig. 3C). In Southern Melanesia, islands in New Caledonia showed clear independent lineages divided by island, with reticulation being evident between Ouvea and Lifou, and a bit weaker between Mare and Grand Terre (Fig. 3B). Similarly, Tanna and Efate showed some reticulation among but were primarily independent lineages. Central Vanuatu islands did not show any evident clustering, but Northern Vanuatu islands (Gaua and Vanua Lava) did.

Water barriers but not continental distances promote population differentiation.

We explored whether water barriers had a stronger impact on population differentiation than continental distances, and found that F_{ST} was greater between insular populations than between continental populations (Fig. 4A; marginal effect at the mean = -0.88; 95% CI [-1.8, -0.034]; Posterior probability = 0.95). F_{ST} increased with distance on islands but not on the continent, although the latter showed wide credibility intervals (Fig. 4B).

Discussion

Silvereyes are excellent island colonisers and have overcome long-distance water barriers multiple times. Given their high dispersal ability, we would expect little population structure. This is the case in continental silvereyes, where despite long distances and ancient biogeographic barriers they show very high levels of connectivity. However, on islands we found a pronounced structure; even geographically close populations were often highly distinct, suggesting that water gaps can be significant barriers even over short distances. Overall these

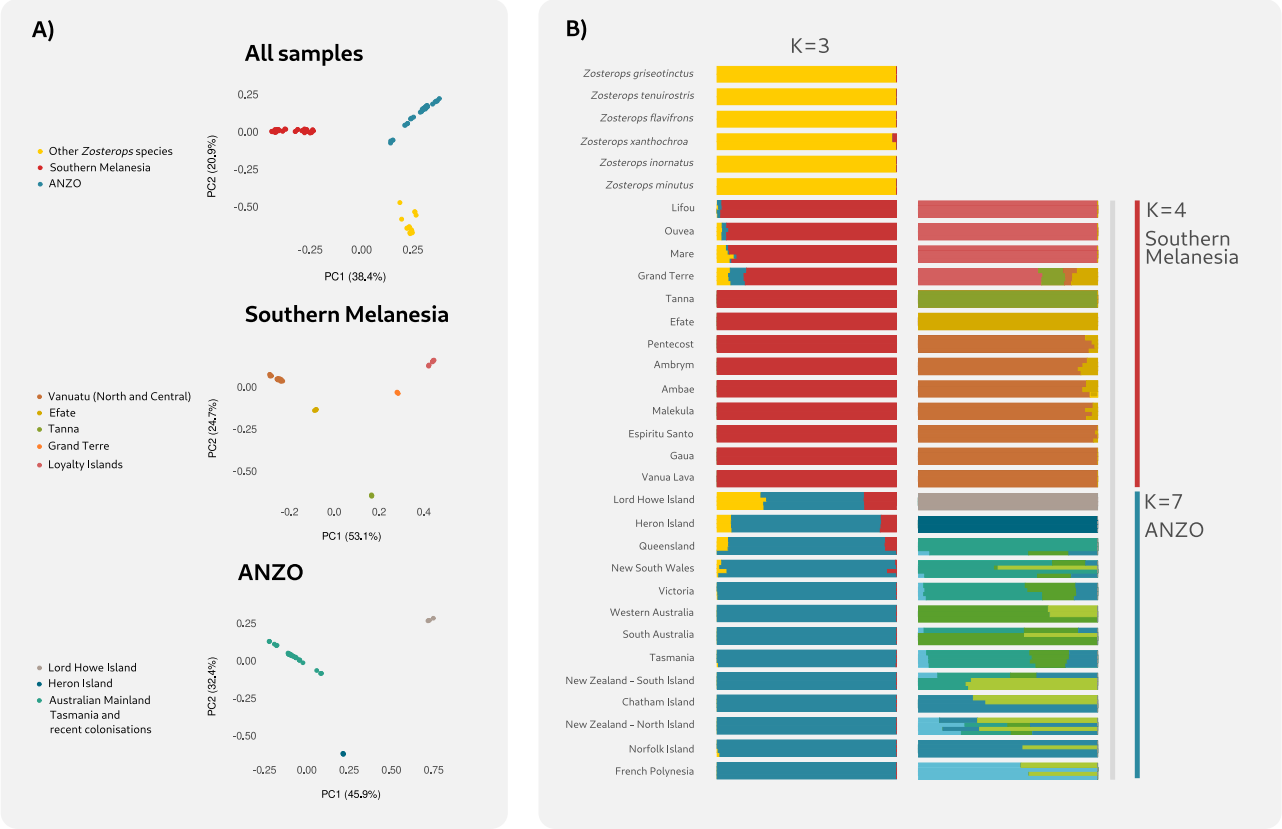


Fig. 2. Population structure of the silvereye clade and other South Pacific white-eyes. A) Genomic PCA plots representing the two axes that explain the most variation (PC1 on the x-axis and PC2 on the y-axis) for the three datasets detailed in the methods section. B) NGSadmix bar plots for: all samples (K=3), Southern Melanesia (K=4), and ANZO (K=7). The represented K is the most likely number of ancestral populations. Each bar is the estimate of the individual's ancestry proportion from each of the assumed ancestral populations.

results indicate that islands play a central role in the diversification of a great speciator.

The evolutionary history of South Pacific white-eyes.

We first discuss the broad evolutionary relationships among the white-eye species in our dataset to provide the framework for resolving finer scale patterns within the silvereye. Our analyses agreed that the silvereye is a monophyletic clade sister to other South Pacific *Zosterops* species and that this divergence event dates approximately 1.5 Ma, during the Pleistocene, when this genus was rapidly colonising a large geographic area throughout the tropics and subtropics, from Asia to sub-Saharan Africa and Oceania (Gwee et al., 2020; Moyle et al., 2009). Our divergence time estimate is very close to both the divergence between the Afrotropical and Australasian *Zosterops,* and *Z. lateralis* from other *Zosterops* species (Martins et al., 2020; Vinciguerra et al., 2023; Wickramasinghe et al., 2017). The overlap in divergence times can be explained by the fact that white-eyes show one of the fastest diversification rates across all vertebrates (Moyle et al. 2009) thanks to their high dispersal ability, colonisation success and subsequent rapid morphological differentiation (Sendell-Price, Ruegg, & Clegg, 2020), and resilience against extinction. Their dispersal ability is especially important for understanding the mechanisms underlying speciation on islands. For example, high levels of dispersal can lead to multiple colonisation events at different points in time leading to species living in sympatry. This idea could explain why two species endemic to Lifou (*Z. minutus* and *Z. inornatus*) are not part of a monophyletic clade. These two species differ in a series of characteristics, with body size being the most striking one: *Z. inornatus* is among the largest of white-eyes (23 g) and *Z. minutus* the smallest (8 g) (Cornetti et al., 2015). According to our phylogeny, *Z. inornatus* must have colonised the island earlier, evolving a large body size as an adaptation to island life, a common pattern seen across *Zosterops* (Estandía, Sendell-Price, Robertson, et al., 2023), and then rapid evolution towards small body sizes via character displacement could have allowed a later arrival to co-exist (Black, 2010; Grant & Grant, 2006). While it is clear that the two species of Lifou white-eye are not closely related, other relationships across the non-silvereye lineage are less clear. For example, *Z. tenuirostris*, endemic to Norfolk Island, appeared both as basal to the New Caledonia species and as sister to *Z. inornatus* in different analyses. Some species of this genus are known to hybridise (Gwee et al., 2020; Manthey et al., 2020) which includes potential admixture across Norfolk Island *Zosterops* (Gill, 1970). If this were the case, gene flow across these species could explain the uncertainty at this node, but further evidence with modelling that accounts for



Fig. 3. Phylogenetic network representing the evolutionary relationships and reticulate evolution. A) Silvereyes and other South Pacific *Zosterops* species, B) Southern Melanesian silvereyes and C) silvereyes from the ANZO cluster, which include continental species that show little clustering, partly due to high levels of gene flow as shown in the TreeMix maximum-likelihood tree on the right. The main directions of gene flow are shown on the map of Australia. Colours represent different populations.

fluctuations in population size is needed.

Phylogenetic relationships and colonisation routes on the silvereye.

The silvereye's likely place of origin is thought to be the Australian mainland, serving as the ancestral source from which all island lineages subsequently emerged, with one of the earliest colonisation events being to Southern Melanesia. Typically, colonisation follows a one-way pattern from continents to islands, although there are exceptions, like the recolonisation of the mainland by *Anolis* lizards (Patton et al., 2021). In the south-west Pacific, colonisation is expected to mainly occur eastward from the larger landmasses, like Australia. The common ancestor could have colonised the mainland through the New Guinea-Australia land bridge that was only covered by the ocean 10,000 years ago (Friedlaender et al., 2008). However, other robust white-eye phylogenies support a more distant relationship between the silvereye and the New Guinea white-eye *Z. novaeguineae,* and a closer relationship with other island species, like *Z. flavifrons* or *Z. explorator*, endemic to Southern Melanesia and Fiji respectively (Oliveros et al., 2021). This evidence raises the possibility that the Australian mainland lineage might have originated from a colonisation event originating in Southern Melanesia or other Pacific islands. However, it is worth noting that the true sister lineage to the silvereye might be extinct, and if it once existed with a distribution overlapping New Guinea and Australia, it could lend support to the hypothesis that silvereyes colonised Australia via the New Guinea-Australia land bridge.

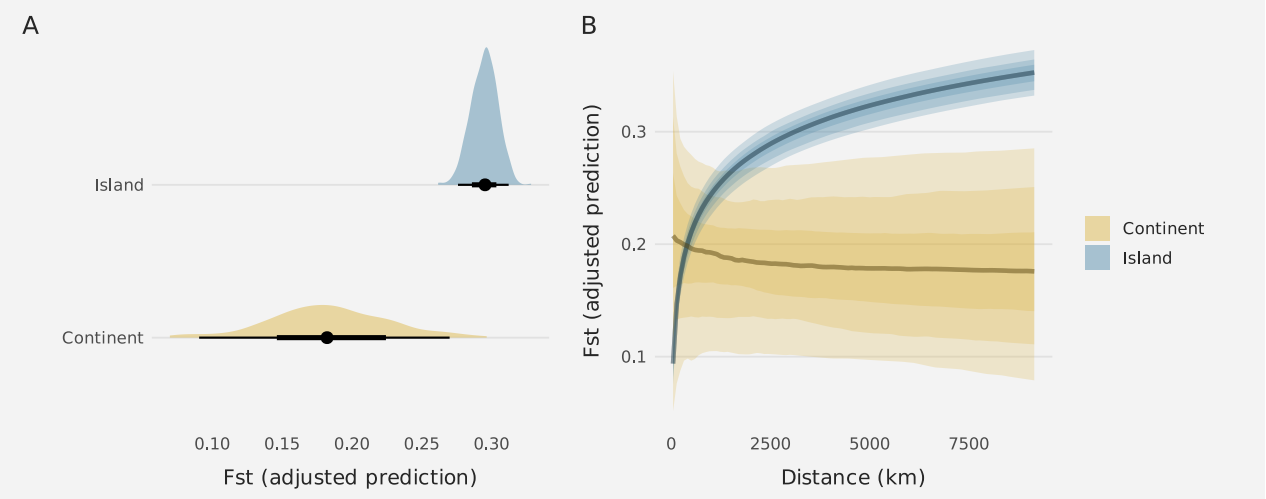


Fig. 4. Population differentiation is stronger between islands. A) Adjusted predictions of F_{ST} on islands and the continent. B) Adjusted predictions of the effect of the distance on F_{ST} levels among continental populations (blue) and islands (yellow). The uncertainty bands are darker for lower values and lighter for higher values, representing 50% 80%, and 95% credibility intervals.

Southern Melanesian silvereyes showed complex phylogenetic patterns, possibly exacerbated by high levels of ILS and gene flow, which can be better represented by phylogenetic networks that allow for the representation of reticulate evolution. We found extensive reticulation across Ouvea and Lifou, aligning with their high morphological similarity. In contrast, Grand Terre and Mare showed less reticulation and were morphologically distinct despite the short distance that separates them and the other Loyalty Islands. Interestingly, Ouvea and Mare silvereyes are classified under the subspecies *Z. l. nigrescens* despite Lifou being in the middle, separating the other two islands and having its own endemic subspecies, *Z. l. melanops*. Visual distances in the PCA capture the geographic distances across Southern Vanuatu islands, as expected under isolation by distance. The most isolated of these islands, Tanna, is also home to the most genetically and morphologically distinct population. In contrast, islands across Central and Northern Vanuatu show very little differentiation, likely due to high levels of gene flow (Clegg & Phillimore, 2010; Estandía, Sendell-Price, Oatley, et al., 2023). The current subspecific taxonomic divisions in silvereyes from Vanuatu do not reflect the genetic structure that we infer here. There are two currently accepted subspecies: *Z. l. vatensis* on Tanna, Efate, Malekula, and Ambrym, and *Z. l. tropica* on Pentecost, Ambae, Espiritu Santo, Gaua and Vanua Lava, even though there is no extensive population differentiation across the archipelago with the exception of Tanna and Efate. However, subspecific taxonomy is complicated and often relies on plumage patterns (Winker, 2021) that are not represented in our analyses.

Lord Howe Island silvereyes *Z. l. tephropleurus* split from the mainland lineage approximately 1 million years ago. This subspecies seems to have been isolated from other silvereye populations as there is no reticulation with any of them. Additionally, individuals from this population have a fully distinct morphology. Interestingly, in the species network, Lord Howe Island shows very mild reticulation with other *Zosterops* species, being closer to them than to either of the two silvereye clades. Possible admixture could have occurred between *Z. l. tephropleurus* and other South Pacific white-eyes, including the robust white-eye *Z. strenuus*, an endemic white-eye species to Lord Howe Island that became extinct in the early 20th century after the introduction of rats (Day, 1981). *Z. l. tephropleurus* is sometimes treated as a full species, however, this would make the silvereye a paraphyletic species by not containing all of its descendants.

Our divergence estimate for Heron Island is much older than we expected (80,000 years ago versus 4,000 that we set as a prior, representing the earliest time that Heron Island was vegetated). Our Bayesian analyses agreed that Heron Island was sister to the rest of the mainland subspecies, but pushed this node too far back. We think that these results are more likely due to an artefact and it can be potentially explained by the combination of the use of nuclear markers instead of mitochondrial markers (e.g. *cytochrome-b*) for which we know the rate of molecular evolution (Arcones et al., 2021), and the strict clock model we used as imposed by SNAPPER. Heron Island was the third island to be colonised from the mainland and despite the fact that many studies have not detected any gene flow (Clegg et al., 2002; Degnan, 1993; Estandía, Sendell-Price, Oatley, et al., 2023; but see Sendell-Price, Ruegg, Anderson, et al., 2020), we find patterns that indicate potential low levels of admixture including very mild reticulation between samples from Queensland and Heron Island. Depending on the method used, Heron Island samples were sister to all mainland samples and recent colonisations, or sister to the Queensland samples. This conflict could be explained by gene flow between both populations. There are reports of mainland silvereyes arriving on Heron Island (Clegg pers. obs.), but they do not necessarily breed.

Mainland birds are indeed highly dispersive: we find extensive connectivity across the eastern coast of Australia, where

all populations have a similar genetic makeup, and individuals cannot be confidently assigned to their original locations. Additionally, there is evidence of strong gene flow between Queensland and New South Wales. Our results are consistent with what we know about silvereye movements in this region since the Tasmanian silvereye and population of *Z. l. westernensis* from Victoria display partial migration, whereby many individuals migrate during winter, a number of which reach southern Queensland (Chan, 1995; Mees, 1969). Tasmania is a continental island that was connected with the mainland by a land bridge until the end of the Pleistocene, approximately 12,000 years ago (Kennett et al., 2018). Despite this barrier, Tasmanian silvereyes continue to display partial migration, which is triggered by changes in light during winter (Chan, 1994). Silvereyes from South Australia share a similar climatic niche, which could potentially trigger migration, but the habitats available northwards are not suitable. Individuals from Western and South Australia did not show any differentiation despite being sampled more than 2,000 km apart and having to overcome substantial biogeographic barriers - the Nullarbor Plain and the Eyrean barrier. At the same time, samples from Victoria and South Australia, collected less than 1,000 km apart, showed some evidence of admixture but clear differentiation. The mechanisms leading to differentiated silvereye mainland populations do not seem to always coincide with geography alone and other mechanisms could be playing a role in determining genetic structure.

We know that the most recent colonisations stem from Tasmania because the arrival of silvereyes to New Zealand is historically documented and the subspecies that arrived is *Z. l. lateralis*, endemic to Tasmania (Mees, 1969). Since the arrival on the South Island of New Zealand, silvereyes colonised Chatham Islands, the North Island of New Zealand and Norfolk Island (Mees, 1969). The French Polynesian populations are a result of a human-assisted introduction consisting of a low number of silvereyes from the South Island of New Zealand in 1937 (Guild, 1938). Our results could not accurately infer the historical trail because this range expansion happened in less than 100 years, suggesting extremely short periods of time between divergence events and high ILS. Similarly, morphological characteristics were too little to be able to distinguish among recently colonised populations, which is likely due to the short amount of time to accumulate enough differences (but see Sendell-Price et al., 2020a). However, we do see in the admixture plot that the genetic makeup of late colonisations resulting from several bottlenecks are less diverse, reflecting the loss of alleles as expected in founder effects (Clegg et al., 2002; Sendell-Price et al., 2021).

Insular population differentiation and the paradox of the great speciators.

Water gaps serve as important barriers for dispersal and gene flow among silvereye populations, leading to higher genetic differentiation. This is not the case for continental populations where, despite long distances and geographical barriers, we see very little genetic structure and no effect of distance on F_{ST} levels. This pattern could be explained in different ways. For example, if island colonisation is rare but there are a few events of successful population establishment, isolated populations could arise that would eventually become separate lineages. However, connectivity patterns on the mainland suggest silvereyes do have an effective ability to disperse. Additionally, overwater dispersal is not entirely rare, as mainland silvereyes regularly reach Heron Island, an island that cannot be seen from the mainland. A more plausible explanation invokes strong selection against dispersal after island colonisation leading to population divergence (Diamond et al. 1976). Individuals on island populations may retain the ability to fly long distances but exhibit a behavioural shift whereby they are reluctant to disperse overwater, a phenomenon known as 'behavioural flightlessness' (Diamond, 1981). This idea fits with previous results that indicate that older and more sedentary populations have experienced changes in dispersal genes when compared with mainland and recently colonised populations (Estandía, Sendell-Price, Oatley, et al., 2023). We see a sharp increase in between-island F_{ST} with distance, suggesting that even short gaps of water can be effective barriers. This is evidenced across southern Vanuatu and the Loyalty Islands where nearby islands show fully genetically distinct populations. However, there are a few exceptions across central Vanuatu where there is evidence of reticulate evolution. In this region, silvereyes have likely been able to maintain populations connected, but there is no obvious reason why. Spatial processes, such as how islands are geographically arranged, could be a third explanation for the observed patterns. This argument does not necessarily require invoking selection against dispersal ability, and it could easily arise with intermediate dispersal (Ashby et al., 2020; Yamaguchi, 2022). Most likely a great speciator pattern would arise from a combination of spatial, abiotic and biotic factors, but the relative importance of each factor is currently unknown. To solve this evolutionary paradox, we should take different approaches, such as theoretical, by building models that help us understand the important parameters, comparative, by exploring the factors driving divergence in numerous great speciators, and empirical, by examining whether island populations show morphological, behavioural, and genetic signatures of reduced dispersal

Conclusion.

This study used genome-wide markers to explore the evolutionary history of a great speciator and whether island and continental settings have promoted population structure and differentiation. We find that islands generally led to higher population differentiation and continental populations were highly connected despite the presence of major biogeographic barriers. We resolved the main deep relationships, but many shallow nodes are unresolved despite the use of a large genomic dataset. Phylogenetic conflict likely arises due to a combination of gene flow and high levels of ILS. Future work should focus on the specific role of gene flow in diversification by tailoring demographic models that account for bottlenecks resulting from island colonisation.

Author contributions

Conceptualization: AE, SMC, BCR; Data Curation: AE; Formal Analysis: AE (Bioinformatics), NMR (Morphology); Funding Acquisition: BCR, SMC; Investigation: AE; Methodology: AE, NMR; Project Administration: AE, SMC; Resources: AE, ATS-P, DAP, BCR, SMC; Supervision: SMC; Visualization: AE; Writing - Original Draft: AE; Writing - Review & Editing: AE, NMR, SMC, DAP, ATS-P, BCR.

Data and code availability

Code to reproduce all analyses is available at https://github.com/andreaestandia/0.0_phylo_silvereye https://github.com/nilomr/silvereye-morphology

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4

Candidate gene polymorphisms are linked to dispersive and migratory behaviour: Searching for a mechanism behind the "paradox of the great speciators"

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Candidate gene polymorphisms are linked to dispersive and migratory behaviour: Searching for a mechanism behind the "paradox of the great speciators"

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Abstract

The "paradox of the great speciators" has puzzled evolutionary biologists for over half a century. A great speciator requires excellent dispersal propensity to explain its occurrence on multiple islands, but reduced dispersal ability to explain its high number of subspecies. A rapid reduction in dispersal ability is often invoked to solve this apparent paradox, but a proximate mechanism has not been identified yet. Here, we explored the role of six genes linked to migration and animal personality differences (*CREB1*, *CLOCK*, *ADCYAP1*, *NPAS2*, *DRD4*, and *SERT*) in 20 South Pacific populations of silvereye (*Zosterops lateralis*) that range from highly sedentary to partially migratory, to determine if genetic variation is associated with dispersal propensity and migration. We detected genetic associations in three of the six genes: (i) in a partial migrant population, migrant individuals had longer microsatellite alleles at the *CLOCK* gene compared to resident individuals from the same population; (ii) *CREB1* displayed longer average microsatellite allele lengths in recently colonized island populations (<200 years), compared to evolutionarily older populations. Bayesian broken stick regression models supported a reduction in *CREB1* length with time since colonization; and (iii) like *CREB1*, *DRD4* showed differences in polymorphisms between recent and old colonizations but a larger sample is needed to confirm. *ADCYAP1*, *SERT*, and *NPAS2* were variable but that variation was not associated with dispersal propensity. The association of genetic variants at three genes with migration and dispersal ability in silvereyes provides the impetus for further exploration of genetic mechanisms underlying dispersal shifts, and the prospect of resolving a long-running evolutionary paradox through a genetic lens.

KEYWORDS
ADCYAP1, *CLOCK*, *CREB1*, dispersal, diversification, *DRD4*, great speciator, islands, *NPAS2*, *Zosterops lateralis*

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1 | INTRODUCTION

Dispersal ability often determines the extent of species ranges and patterns of population divergence and speciation (Birand et al., 2012; Suárez et al., 2022). Highly dispersive taxa tend to have larger geographic ranges (Alzate & Onstein, 2022; Lester et al., 2007; Sheard et al., 2020) and more opportunities to exchange genes among populations in different parts of their range, inhibiting diversification (Birand et al., 2012; Gillespie et al., 2012; Lack, 1969, but see Cardillo et al., 2003). In addition, the spatial scale of speciation has been linked to variation in gene flow across a range of taxa (Kisel & Barraclough, 2010), and reduced gene flow during early stages of divergence is central to many speciation models (Coyne & Orr, 1998; Grant & Grant, 2008; Hedrick, 1983; Mayr & Diamond, 2001; Slatkin, 1987).

Great speciators are those species that inhabit multiple islands reached by overwater dispersal events, suggesting excellent colonization ability, yet display multiple morphological subspecies (Diamond et al., 1976; Manthey et al., 2020; Moyle et al., 2009; Pedersen et al., 2018). This concept is applied to several subspecies-rich birds from Melanesia and the South Pacific, including representatives within the white-eyes, kingfishers, and cicadabirds (Mayr & Diamond, 2001). A common explanation to reconcile broad island distributions with phenotypically diverse taxa is that dispersal traits are strongly selected against in each newly established island population (Diamond, 1981). Selection may reflect the individual energetic cost of this behaviour relative to the benefits of remaining (Bonte et al., 2012; McNab, 2002) or the idea that once most islands are filled, the chance of successful overwater dispersal is diminished (Diamond, 1970). However, it is still unclear how these relatively rapid, post-colonization changes in dispersal propensity could arise.

Variation in dispersal propensity is largely determined by differences in a combination of morphological, physiological and behavioural traits (Matthysen, 2012). In birds, the types of morphological changes that indicate a reduced dispersal ability include a more rounded wing (as indicated by a lower hand-wing index) (Kipp, 1959) and a shift to a more graviportal body plan (reduced flight muscles and longer legs) (Wright et al., 2016). However, among the great speciators, the shift from dispersive to sedentary forms may primarily involve behavioural rather than morphological or physiological changes, at least initially (Diamond, 1981). This is termed "behavioural flightlessness", a reluctance to disperse (especially across water), despite the maintenance of normal wings and flight. Hence, focusing solely on morphological proxies for dispersal ability that take time to change risks missing an essential part of the early evolutionary process.

A useful but relatively unexplored framework to understand behavioural flightlessness and subsequent divergence considers the links between dispersive behaviours and personality traits (Ingley & Johnson, 2014). Dispersal (single movement to a new area) is a complex behavioural syndrome that co-varies with other personality traits: highly dispersive taxa often show increased levels of boldness, aggression and exploration (Botero-Delgadillo et al., 2019; Duckworth & Badyaev, 2007; Duckworth & Kruuk, 2009). For example, highly aggressive Western bluebird (*Sialia mexicana*) individuals were found to be more dispersive than their less aggressive counterparts (Duckworth & Badyaev, 2007; Duckworth & Kruuk, 2009); and dispersive great tit (*Parus major*) individuals had a greater exploration rate than non-dispersive individuals (Korsten et al., 2013). Migration (seasonal movement) is also associated with personality in animals, in particular in those species that display partial migration (Nilsson et al., 2014) Migratory individuals within a species tend to be bolder than non-migratory ones (Chapman et al., 2011). For example, European Stonechat (*Saxicola rubicola*) migrant males were more territorial than resident males (Marasco et al., 2011) and resident blue tits (*Cyanistes caeruleus*) were more neophobic than migrant individuals (Nilsson et al., 2010). In addition, migratory taxa tend to disperse further (Paradis et al., 1998) within species (Kelly et al., 2016; McCaslin et al., 2020) and across species (Sheard et al., 2020). Off-course migration can result in the founding of new populations, as vagrancy is linked to exploration in migratory birds (Zawadzki, 2021).

Both quantitative and molecular genetic studies provide evidence of a genetic influence on migratory and dispersive behaviours (Liedvogel et al., 2011). These behaviours often have significant narrow sense heritability, often greater than 0.4 (Dochtermann et al., 2019). Additionally, at the molecular level, genetic variation for migration, dispersive behaviour and personality traits are well documented (Bubac et al., 2020), including the identification of a number of candidate genes underlying personality traits such as tendency for boldness or exploratory behaviour (Canestrelli et al., 2016; Fidler et al., 2007; Ruegg et al., 2014; Steinmeyer et al., 2009), and migratory behaviour (Bazzi et al., 2015; Johnsen et al., 2007; Peterson et al., 2013; see Le Clercq et al., 2023 for an extensive review). Changes in genetic variation at these candidate genes may act as a genetic switch catalysing population-level shifts in dispersive behaviour.

To date, six candidate genes are thought to contribute to a migratory phenotype, dispersive behaviour and personality traits in at least some bird species (Bubac et al., 2020; Table S1). In four of these genes, "adenylate cyclase activating polypeptide 1 gene" (*ADCYAP1*; Mettler et al., 2015; Saino et al., 2015); the polyglutamine repeat region of the "circadian locomotor output cycles kaput gene" (*CLOCK*; Johnsen et al., 2007; Peterson et al., 2013; Saino et al., 2015), the "neuronal PAS domain protein 2" (*NPAS2*; Chakarov et al., 2013; Bourret & Garant, 2015), and the "cAMP responsive element binding protein 1" (*CREB1*; Bazzi et al., 2017), microsatellite allele length variation is associated with migratory-related traits across a variety of avian taxa (Table S1). The other two genes ("dopamine receptor D4" [*DRD4*] and "serotonin transporter" [*SERT*]) show associations between single nucleotide polymorphisms (SNPs) and differences in avian personality and migratory propensity (Riyahi et al., 2017; Timm et al., 2019; Sauve et al., 2021; Table S1).

The Zosteropidae family (white-eyes, yuhinas and allies) consists of 142 species (Clements et al., 2019) many of which are highly

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dispersive as evidenced by colonization of numerous oceanic islands throughout the Indian and Pacific oceans, along with the broad continental distributions of some species (Clegg et al., 2002; Mees, 1969). This family shows one of the highest per-lineage diversification rates for vertebrates (Moyle et al., 2009) and divergence can occur even across minor geographic barriers (e.g. water gaps of just 2 km) (Bertrand et al., 2014; Cowles & Uy, 2019; Glor, 2011; Manthey et al., 2020; Mees, 1969). A particularly interesting species within this family is the silvereye (*Zosterops lateralis*), considered a great speciator having multiple subspecies (17 morphological subspecies; Clements et al., 2019) with a wide natural distribution—including the Australian mainland and Tasmania, the North and South Islands of Aotearoa New Zealand (henceforth New Zealand), outlying oceanic islands of Australia and New Zealand, and the archipelagos of New Caledonia, Vanuatu, and Fiji (Figure 1a). Silvereyes also display a variety of gene flow potentials: the Tasmanian subspecies (*Z. l. lateralis*) is a partial migrant (Mees, 1969); those in the central Vanuatu archipelago display high levels of outgoing gene flow, while more peripheral populations have high levels of incoming gene flow (Clegg & Phillimore, 2010); and others, such as on Heron Island and Lord Howe Island in the southern Great Barrier Reef, are sedentary and genetically isolated (Sendell-Price et al., 2020).

Here, we assess variation in six personality-related candidate genes in the silvereye at three different levels: individuals, populations and subspecies. Our aim is to determine whether there are signatures consistent with a genetic switch that would explain rapid shifts in dispersal, leading to reduced gene flow and subsequent increased opportunity for divergence. We ask (i) if candidate gene variation follows patterns of neutral genomic divergence, that reflect drift and gene flow processes only; (ii) if candidate gene variation is associated with dispersal propensity among a range of dispersive and non-dispersive populations of silvereye, considering time since colonization; and (iii) if candidate gene variation is correlated with individual migratory status in the partially migratory Tasmanian population.

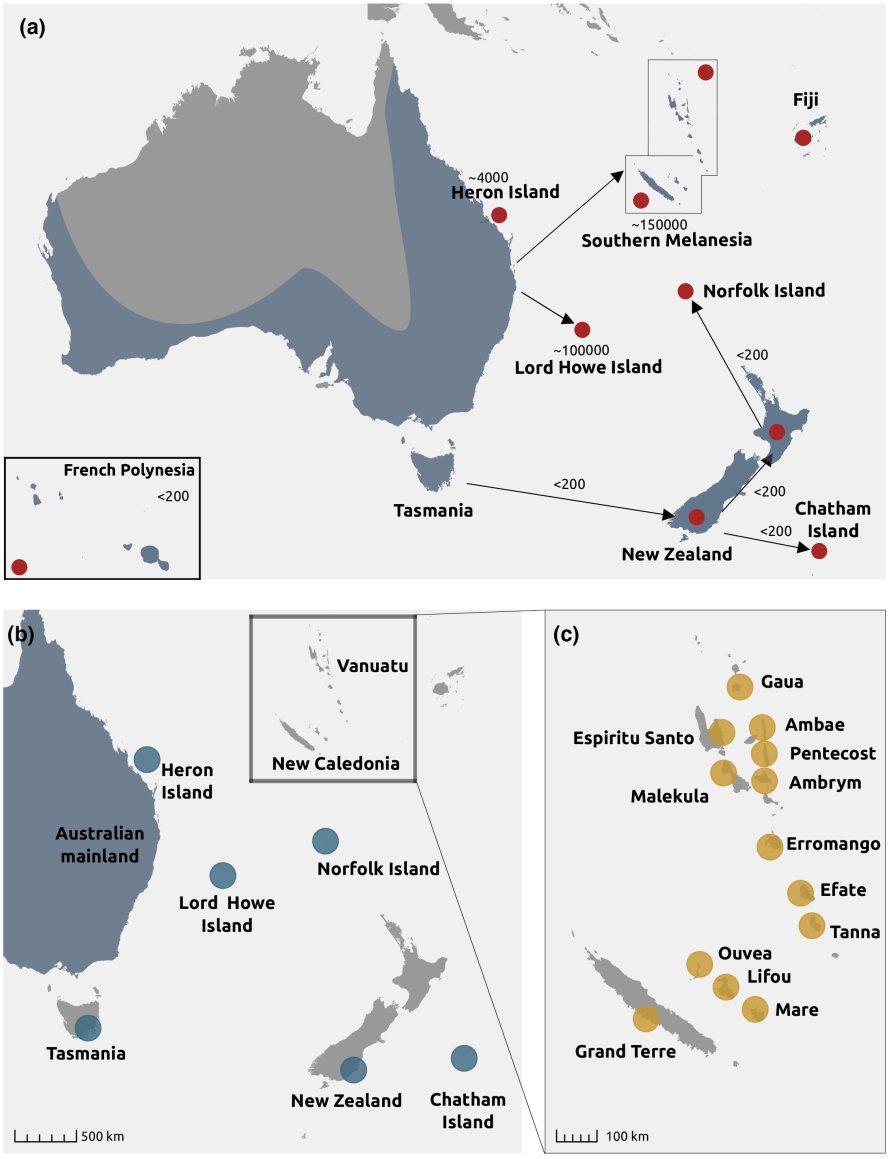


FIGURE 1 (a) Distribution range of the silvereye highlighted in blue. Red dots represent single islands or archipelagos where multiple islands have been colonized from the Australian mainland or Tasmania. Approximate colonization times are shown. French Polynesia is shown as inset; Locations of silvereye populations sampled for candidate gene variation: (b) ANZO cluster (c) SM populations.

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2 | METHODS

2.1 | Sampling and DNA extraction

Silvereye blood samples were collected between 1996 and 2016 from 20 sites in eastern Australia and New Zealand, including outlying islands, henceforth ANZO (Figure 1b), and Southern Melanesia (New Caledonia and Vanuatu) referred to henceforth as SM (Figure 1c; Table S3). The sampling included putative Tasmanian winter migrant individuals captured in the Australian mainland states of Queensland and New South Wales. Individuals were assigned as Tasmanian migrants based on plumage differences: resident silvereyes (subspecies *Z. l. cornwalli*) exhibit bright yellow throat and grey flanks, while migrant individuals (subspecies *Z. l. lateralis*) exhibit white-to-pale-yellow throat and dark buff flanks (Figure S1; Higgins et al., 2006). Birds were caught in mist-nets or traps and blood samples were taken via venipuncture of the brachial wing vein and stored in 90% ethanol or lysis buffer (10 mM EDTA pH 8.0, 10 mM TRIS–HCl pH 8.0, 20 mM NaCl, 1% SDS, Seutin et al., 1991).

2.2 | Candidate microsatellite genotyping

We extracted DNA from 422 samples using a standard chelex protocol (Walsh et al., 1991) and amplified four microsatellite candidate genes (*CREB1* [$n = 247$], *CLOCK* [$n = 258$], *ADCYAP1* [$n = 329$], and *NPAS2* [$n = 375$]), (Table S3) with the primers from Steinmeyer et al. (2009) using a touchdown polymerase chain reaction (PCR) protocol and fluorescent labelling (VIC and FAM) using M13 tags (Schuelke, 2000). Fluorescent labelling with VIC and FAM was done in multiplex PCR reactions containing two loci each (VIC: *CLOCK* and *ADCYAP1*; FAM: *CREB1* and *NPAS2*); final reaction volume of 3 µL contained 10 ng DNA, 1 µL Type-it Master Mix (Qiagen), 0.17 µM of either FAM or VIC, and 0.01 µM forward M13-labelled primer and 0.05 µM reverse primer for each locus.

Thermal cycling consisted of an initial denaturation step of 95°C for 15 min, eight cycles of 94°C for 30 s, an annealing temperature of 60°C, reduced by 1°C each cycle, for 90 s and a 72°C extension step for 60 s, then 25 cycles of 94°C for 30 s, 52°C for 90 s, and a 72°C for 60 s, and a final extension of 60°C for 30 min. VIC and FAM multiplexes were pooled for each sample and allele sizes for the candidate gene microsatellites were determined in relation to LIZ600 size standard on an ABI 3730xl DNA Analyser and scored using the Microsatellite Plugin within Geneious 2020.1 (http://www.geneious.com, Kearse et al., 2012). We tested for the deviations from Hardy–Weinberg Equilibrium, allelic richness and heterozygosity using the R package *diverRsity* (Keenan et al., 2013).

2.3 | DRD4 and SERT sequencing

We targeted one region of *DRD4* ($n = 34$), encompassing intron 2 and exon 3, and part of intron 3 (1480 bp) following Fidler et al. (2007). For *SERT* ($n = 100$), we amplified part of the promoter region (508 bp) following Holtmann et al. (2016). Each 25 µL PCR reaction was comprised of approximately 10 ng DNA template, 1.0 unit BioTaq (Bioline USA Inc.), 1× BioTaq reaction buffer, and 0.5 µM each forward and reverse primer, 200 µM dNTPs, 1.5 mM $MgCl_2$, and made up to volume with Milli Q water. The PCR reaction profiles for both *DRD4* and *SERT* fragments consisted of an initial denaturation step of 94°C for 3 min, followed by 10 cycles of 94°C for 3 min, a touchdown step at 65°C for 30 s, and an extension step of 72°C for 2 min. This was followed by 25 cycles of 94°C for 30 s, 55°C annealing step for 30 s, an extension step of 72°C for 2 min, and a final extension step of 72°C for 10 min. We purified PCR products using Acroprep 96 filter plates (Pall Corporation) following the manufacturer's protocol, then Sanger sequenced with forward and reverse primers on an ABI 3730xl DNA Analyser (Genetics Analysis Service, Otago University, NZ). We aligned the sequences using MUSCLE (Edgar, 2004) in Geneious version 2020.1 and identified SNPs and insertions/deletions (indels). For *DRD4*, we aligned sequences to the great tit *DRD4* gene sequence (GenBank accession no.: DQ006801.1; Fidler et al., 2007) and for SERT, we used the dunnock (*Prunella modularis*) sequence (Genbank accession no.: KT967954.1; Holtmann et al., 2016). We called SNPs with a minimum minor allele frequency (MAF) of 0.05.

2.4 | Gene flow estimates

As a proxy of population dispersiveness, we estimated outgoing gene flow rates from each population. We used whole genome sequences published by Estandía et al. (2023) representing 336 of the 422 individuals included in the present study to examine population structure patterns in NGSadmix (Skotte et al., 2013), a module implemented in ANGSD (Korneliussen et al., 2014). All populations are represented in the whole-genome dataset except Erromango. We obtained a BEAGLE file containing genotype likelihoods and created a subset of 10 000 unlinked SNPs picked at random after applying filtering for a MAF of 0.05. We ran NGSadmix with a range of genetic clusters (k), from 2 to 20. We selected the best k for our dataset based on the mean estimate likelihoods, which indicated $k = 2$ as the optimal number of clusters, corresponding to ANZO and SM groupings. We re-ran NGSAdmix in each of the main cluster to explore potential substructuring. We visualized clustering patterns using a custom R script.

We generated a covariance matrix in PCAngsd (Meisner & Albrechtsen, 2018) using the BEAGLE file. Because not all individuals were screened for all candidate genes (e.g. 375 individuals for *NPAS2* but 258 for *CLOCK*), and not all individuals in the candidate gene and whole-genome dataset coincide, we produced a population-level covariance matrix. From this, we calculated contemporary rates of gene flow among *Z. lateralis* populations for each of the two clusters (ANZO and SM) separately using BA3-SNP (Mussmann et al., 2019), an extension of BayesAss that allows SNPs as input. To ensure that our gene flow

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estimations were not a biased product of the SNP selection, we created five independent datasets of randomly selected 10000 SNP as input. BayesAss employs a Bayesian approach with Markov chain Monte-Carlo (MCMC) sampling to estimate migration rates defined as the proportion of immigrants from the donnor population to the sink population. We adjusted delta values for migration rates (m), allele frequencies (a) and inbreeding coefficients (f) using Autotune (Mussmann et al., 2019) to ensure that parameter space sampling acceptance rate was between 20% and 60% (Wilson & Rannala, 2003). We ran the program for half a million iterations, discarding the first 10% as burn-in. We estimated the 95% credible sets by calculating the mean $\pm$ 1.96 $\times$ Standard deviation (SD).

To assess model convergence, we compared results from 10 replicate runs each with a different random starting seed. We considered runs to have converged on a similar solution if gene flow estimates were within 0.005 (0.5%) across runs. We averaged the parameter estimates of the runs for subsequent analyses.

We explored population structure within the partial migrant population by doing a genomic PCA with PCAngsd and decomposed the covariance matrix into eigenvalues. We plotted the two first PCs to visually explore any emerging structure.

2.5 | Candidate gene association analysis

For all candidate genes, we assessed variation among populations using the R package *brms* (Bürkner, 2017). Individual mean microsatellite length was set as the response variable and population was set as a categorical explanatory variable. In the case of *SERT* and *DRD4*, the response variable was coded as 0 or 1 representing whether the individual carried the most frequent nucleotide or the variant, and we used a Bernoulli family for binary outcomes. For the two microsatellite candidate genes that showed obvious within and between population variation in length (*ADCYAP1* and *CREB1*) we tested whether population age and dispersal propensity could explain this variation by running Bayesian linear mixed models in *brms*. *CREB1* showed a clear distinction in allele lengths between a grouping consisting of the Australian mainland, Tasmania and recently colonized islands versus old island populations. Because of this structure (that does not completely align with ANZO and SM neutral structure groupings), we also applied a broken stick regression model for *CREB1* only to test whether including a single change point would improve predictive performance over a null model and a linear model using *mcp* (multiple point change) (Lindeløv, 2020). *NPAS2* and *CLOCK* displayed little among population variation in average allele length, hence we did not apply Bayesian linear mixed models to the whole population set. However, *CLOCK* showed variation within the partial migrant population of Tasmania, so we tested whether individuals that migrated to the mainland had different *CLOCK* lengths to those that over-wintered in Tasmania. The Tasmanian sample was restricted to 14 winter-caught birds (non-migrants) as the resident summer population includes a mix of migrants and non-migrants that cannot be phenotypically distinguished. The 'migrant' group consisted of those caught at Australian mainland sites in winter that were phenotypically identified as Tasmanian migrants (26 individuals).

For Bayesian linear mixed models (*CREB1* and *ADCYAP1*) and broken-stick regression models (*CREB1*), the following population-level (fixed) parameters used were:

1. Dispersal Index (DI): the sum of each outgoing gene flow estimate (g) from island i into island j (where 95% credible interval did not overlap with zero), multiplied by the geographic distance between the islands (d) Equation (1). The latter helps to account for differences in geographic opportunity for dispersal; for example, a geographically isolated island population that has moderate outgoing gene flow to few far islands would score higher than a centrally located island population with moderate outgoing gene flow to many close islands. DI was scaled from 0 (non-dispersive) to 1 (maximally dispersive).

$$\mathrm{DI} = \sum g_{i \rightarrow j}^{*} d_{i \leftrightarrow j} \quad (1)$$

2. Age (Table S2): population ages for New Zealand, Chatham Island and Norfolk Island are known from historical records (c. 190 years; Clegg et al., 2002; Mees, 1969); for Heron Island, the population age was set as a maximum of 4000 years based on length of time the island has been vegetated (Clegg et al., 2008); and molecular estimates for the remaining ancient populations were taken from a time-calibrated gene tree (Black, 2010).

The null model represents the starting hypothesis that mean *CREB1* or *ADCYAP1* length differs between populations (which was set as group-level parameter or random effect) but does not change as a function of increasing dispersal propensity or time since colonization. We controlled for population structure using the PCAngsd population-level covariance matrix. As *mcp* does not permit incorporation of distance matrices, we used a categorical variable that classified each individual according to its membership in one of the two population genetic clusters (ANZO or SM) identified from the top-level NGSadmix analysis.

Our models used a Gaussian distribution and application of the default link function for all *mcp* (*CREB1* only) and *brms*:

(i) Differences in length among populations for each candidate microsatellites
(ii) Bayesian linear mixed models:

1. migration: *CLOCK* only
2. population age and DI: *CREB1* and *ADCYAP1* only.

We set weakly informative priors for the intercepts of the segments before and after the change point (*mcp*). The same prior was applied

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for both *CREB1* length intercepts due to the lack of a priori information about a positive or negative correlation of mean *CREB1* length with population age and dispersal index. Priors for both intercepts were centred at 550 bp with a SD of 20 ($N(550, 20)$), based on known mean and variance of *CREB1* lengths in other passerines (Steinmeyer et al., 2009). For the *brms* linear mixed model, we set weakly informative priors with a normal distribution centred on 0 with a standard deviation of 20 for the DI and population age coefficients. This prior does not assume an increase or decrease in length, but incorporates the prior information that changes will not be greater than 20 bp given that *CREB1* tend to vary less than 10 bp in other birds (Bazzi et al., 2017; Bourret & Garant, 2015). We performed prior predictive checks, where data was generated according to the specified prior predictive distributions in order to assess their suitability (Gabry et al., 2019). Since dominance of the longer allele occurs for some candidate genes that show Poly-Q polymorphism (e.g. Saino et al., 2015), we analysed all the models with the longer allele as the response variable.

For both the *brms* and *mcp* analyses, we used MCMC with four chains of 4000 iterations each, including a warm-up of 400 iterations. We evaluated convergence via visual inspection of the MCMC trace plots, checking that the ESS > 200 and the R values for each parameter ($R = 1$ at convergence). To evaluate model performance, we compared our fitted models with an null model using leave-one-out cross-validation (LOO), a robust, fully Bayesian model selection approach (Vehtari et al., 2017).

3 | RESULTS

3.1 | Population genetic structure and gene flow

NGSadmix analysis of WGS data supported two main genetic clusters ($k = 2$): Cluster 1 comprised Australia, New Zealand and outlying island populations (ANZO), and Cluster 2 comprised Vanuatu and New Caledonia populations in Southern Melanesia (SM) (Figure 2; Figure S2; Table S4). Other values of k also had high likelihoods (Table S4); $k = 3$ indicated sub-structuring within ANZO due to separation of Heron Island and Lord Howe Island from other populations (Figure S2; see also $k = 2$ in the ANZO analysis Table S4.1); $k = 4$ indicated sub-structuring within the SM cluster, primarily separating New Caledonia from Vanuatu populations, with the Vanuatu island of Tanna showing some affiliation with New Caledonia (Figure S2, see also $k = 2$ in the SM analysis Table S4.2). Increasing the value of k resulted in structuring based on single islands. These population genetic patterns were consistent with those that emerged from the covariance matrix (Table S5; Figure S3).

The independent BayesAss runs conducted to quantify the degree of migration rates agreed on the patterns of gene flow (Table S6). Of 42 pairwise comparisons within the ANZO cluster, and 132 in the SM cluster, nine and five respectively had estimates for which the credibility intervals did not overlap with zero. Within

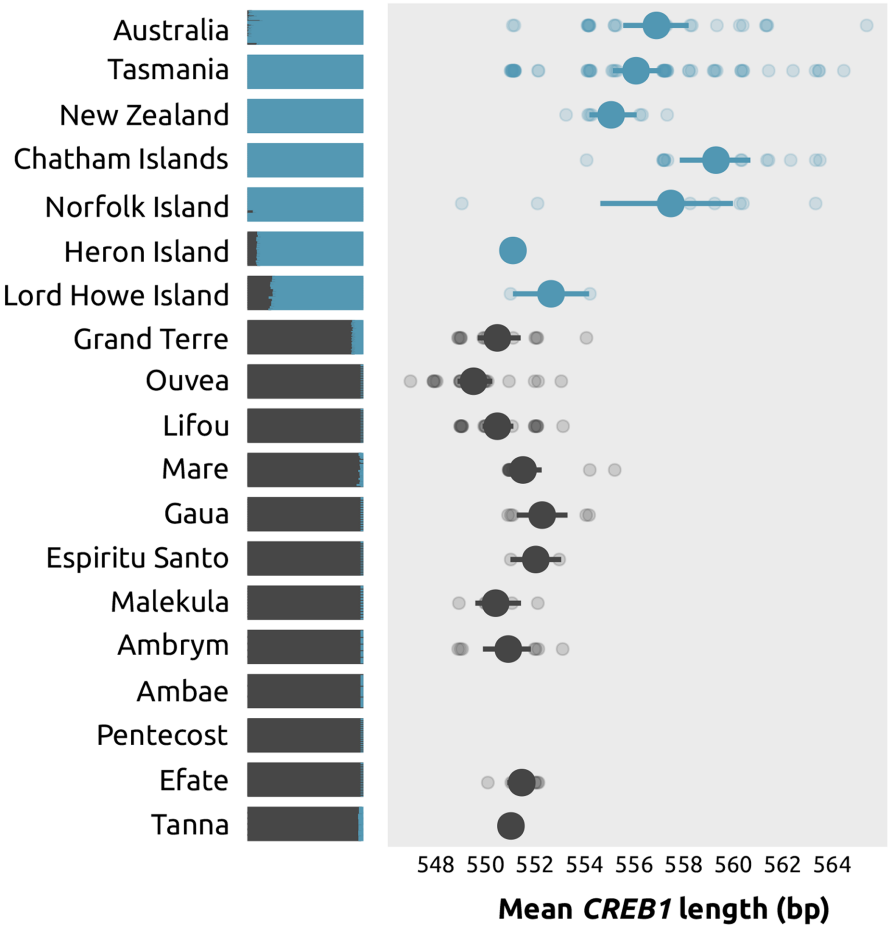


FIGURE 2 NGSadmix plot ($k = 2$) showing a split between ANZO and the SM populations. The average *CREB1* allele length per population indicates a reduction in length for Heron and Lord Howe Islands, and all populations in the SM cluster. *CREB1* was not screened for Ambae and Pentecost samples.

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ANZO, this was primarily seen in relatively high outgoing gene flow estimates from Tasmania, and within SM, moderate outgoing levels from central islands of Pentecost and Espiritu Santo.

We did a genomic PCA with migrant and resident individuals and found that the two first PCs did not separate discrete clusters containing migrants and residents (Figure S4), suggesting a lack of general genome-wide divergence.

3.2 | Variation in candidate microsatellites, DRD4 and SERT

Mean and individual allele lengths for microsatellite candidate loci are shown in Figures 2 and 3. *CREB1* showed longer mean lengths for the Australian mainland, Tasmania (migrants and non-migrants), and the recently colonized populations of New Zealand, Chatham Island, and Norfolk Island (Figure S4; Table S7.1). Heron Island, Lord Howe Island and all Southern Melanesian populations displayed shorter allele lengths on average.

CLOCK was monotypic in the majority of populations (Figure 3b; Table S7.2). Compared to Tasmanian resident silvereyes, Tasmanian migrants showed longer allele lengths. Migrant individuals had long *CLOCK* variants (allele lengths of 289 and 291) not observed in any winter-caught Tasmanian birds (i.e. residents) (Figure 4a), or any other population except for a Heron Island individual. *NPAS2* showed some variation across populations but similar mean values for populations in the ANZO cluster, with the exception of Lord Howe Island (Figure 3d; Table S7.3). However, most SM populations were not variable at this locus, with the exception of peripherally located islands of Gaua, Efate and Tanna in Vanuatu. Variation between and within populations for *ADCYAP1* was evident for all populations (Figure 3c; Table S7.4).

We identified 10 *DRD4* SNPs, seven of which were non-synonymous and three synonymous. Five of them showed significant differences across populations but only SNP at position 83 (a non-synonymous substitution) displayed consistent differences between ANZO and SM clusters (Table S7.5). SM individuals exclusively carried adenines, resulting in production of lysines, while those from New Zealand and Chatham Island only carried guanines producing arginines. Tasmanian residents and Tasmanian migrants had both nucleotides represented. We found one SNP and an INDEL in *SERT* but none were significantly different in frequency among populations.

3.3 | Candidate gene association tests

Tasmanian residents showed a decrease of 1 bp ([−0.05, −1.94] 95% credibility intervals) in *CLOCK* relative to migrants (Figure 4b; Table S8). Mean *CREB1* variation was better explained by a single change point model than the null model or a linear regression model

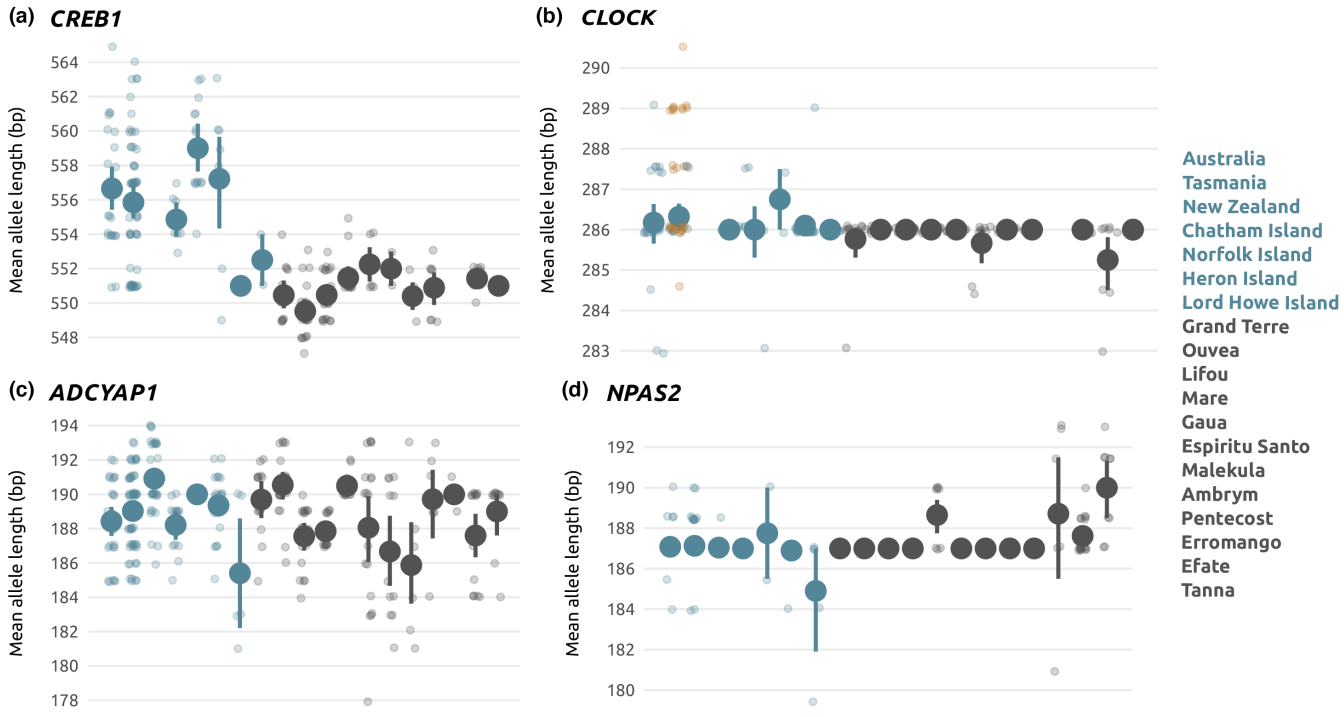


FIGURE 3 Individual mean allele lengths (shadowed dots) with mean and standard error for each population (large dots) for the four microsatellite candidate genes. Blue and grey dots correspond to two population genetic clusters identified from admixture analysis. (a) *CREB1* is longer in the source populations (Australia and Tasmania), and recently colonized populations (New Zealand, Chatham Islands and Norfolk Island) compared to older populations (Heron Island, Lord Howe Island, and SM populations). (b) Migrant individuals highlighted in orange have longer allele lengths at *CLOCK*. (c) *ADCYAP1* shows extensive variation, including shorter lengths in Lord Howe Island and Ambrym and longer lengths in New Zealand, Ouvea and Gaua. (d) *NPAS2* shows little variation with southern Vanuatu populations (Erromango, Efate and Tanna) showing an increased mean allele length.

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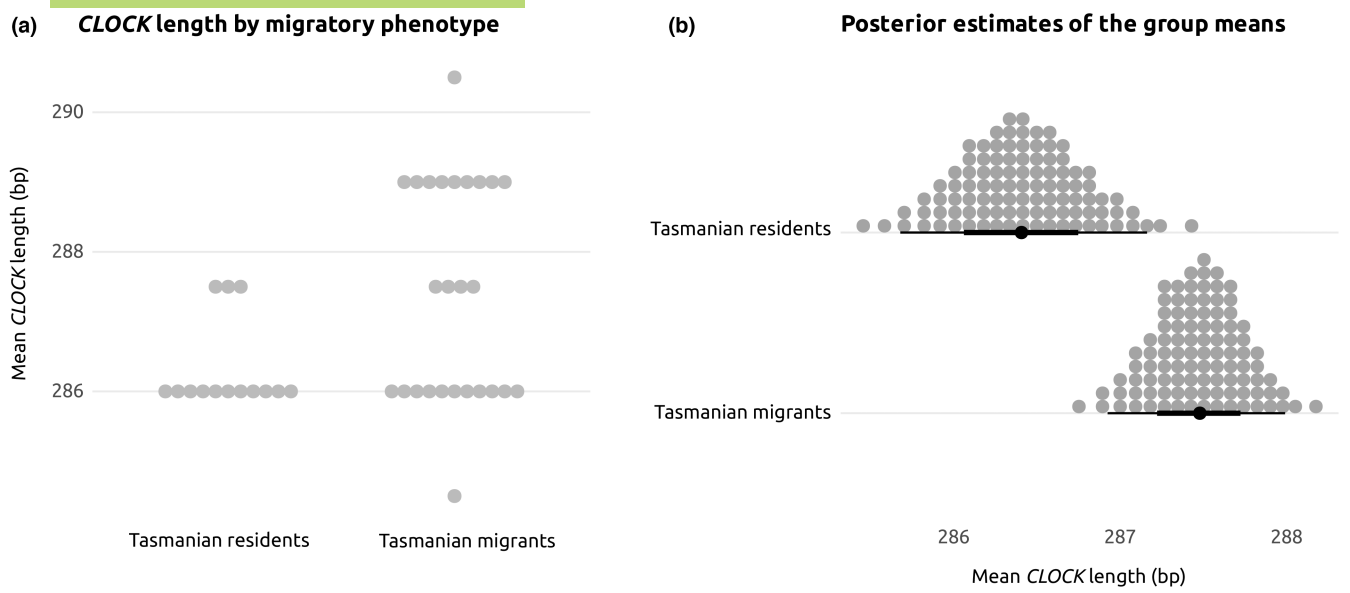


FIGURE 4 (a) Individual *CLOCK* lengths for Tasmanian residents and Tasmanian migrants (Tasmanian silvereyes caught in winter on the Australian mainland). Tasmanian migrants show longer allele lengths. (b) Posterior estimates of the group means obtained from the *brms* model. Migrant individuals have a 1 bp increase when compared with residents.

(Figure S4; Tables S9.1–S9.2). Time since colonization and DI failed to explain the variability in *ADCYAP1* (Tables S10.1–S10.2). All models performed with the longer allele yield the same results.

The single change point model indicated that mean *CREB1* length decreased six base pairs with increasing population age, however the timing of this change had high uncertainty (Intercept 1 (before change point) = 552.462, Intercept 2 (after change point) = 557.494; Table S10.1; Figure 5a). The posterior probability density of change point ranged between 200 and 4000 years ago when we see the step reduction in allele length in the Heron Island population (maximum age 4000 years, Clegg et al., 2008), a reduction that is observed in other older populations (Lord Howe Island and Southern Melanesian populations). The exception is Tasmania, an evolutionarily old population with long average *CREB1* length. *CREB1* decreased five base pairs with decreasing DI (Intercept 1 = 551.22, Intercept 2 = 556.68; Table S10.2; Figure 5b). The posterior probability density of change point for DI ranged from Chatham Island to Espiritu Santo, an island in Vanuatu that shows low levels of outgoing gene flow into nearby islands.

4 | DISCUSSION

Rapid changes in dispersal propensity could help to explain why taxa present on many islands show high levels of phenotypic diversity—the paradox of the great speciators. Quantifying variation in six candidate genes thought to underlie dispersal and migratory behaviour in populations of Australian mainland and island-colonizing silvereyes has revealed three key results that hint at a role for allelic changes as a mechanism underlying this paradox. First, at the population level, we found that *CREB1* allele length variation was associated with time since colonization and dispersal propensity, with recently colonized populations and those with high dispersal tendencies carrying long alleles. Second, at the individual level, we found allele length variation in *CLOCK* associated with migratory status when comparing Tasmanian residents (shorter) and Tasmanian migrants (longer). Third, more limited data from a single *DRD4* SNP suggested that recently colonized populations carried guanines, evolutionarily older island populations carried adenines, and Tasmanian migrants and non-migrants carried both nucleotides. Together, these genes are likely to provide useful signatures of behavioural shifts in dispersal propensity in other silvereye populations, and possibly closely related species, though whether any of these genes act as a genetic switch remains unclear.

In the silvereye populations studied here, *CREB1* showed a disjunct pattern of allele sizes: longer in the mainland, the partial migrant Tasmanian population, and all of the recently colonized populations and shorter in island populations colonized thousands, or hundreds of thousands of years ago. This cannot be explained by population genetic groupings, as both the Heron Island population (up to 4000 years old) and Lord Howe Island population (over 100 thousand years old) (Clegg et al., 2002; Sendell-Price et al., 2020) had shorter *CREB1* allele lengths, yet clearly fell in the ANZO population genetic cluster that includes all of the long-allele-length populations. The Heron Island samples were monomorphic for a unique (551 bp) allele, and we cannot rule out that it may be fixed entirely due to drift in this small population (of ~300 breeding birds, McCallum et al., 2000). However, all of the older colonizations converge in a reduction in length, suggesting that it is unlikely that just by neutral processes each of these independent colonizations show a change in length in the same direction. While most of the island populations were not monomorphic, they showed a decrease in variance and a shift in allele lengths. Populations did not have a fixed genotype in young (dispersive genotype) versus old colonizations (sedentary

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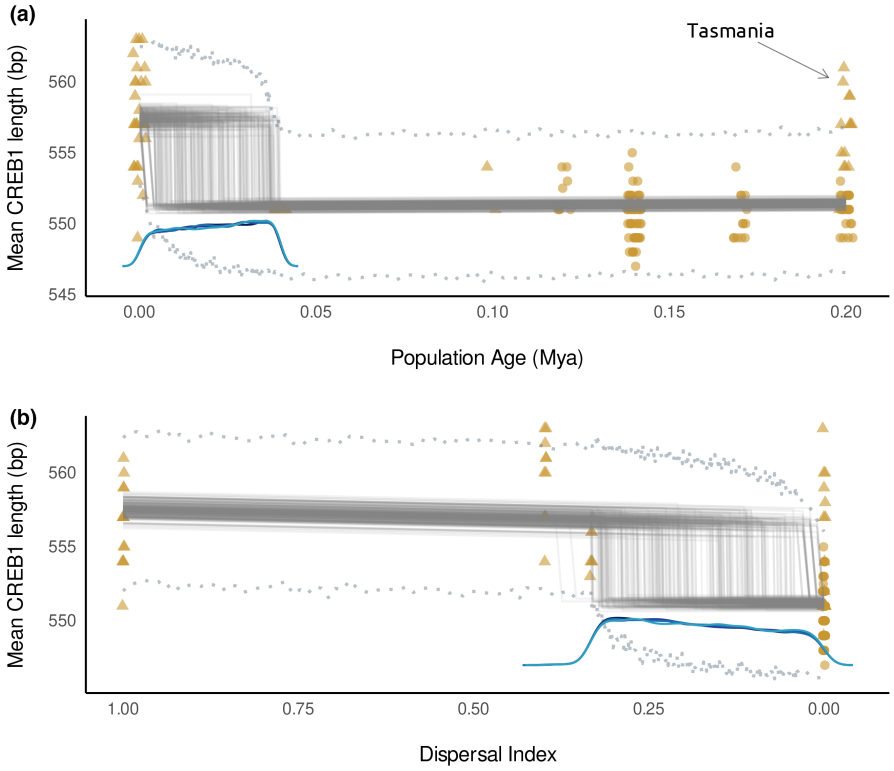


FIGURE 5 Broken-stick regression model (*mcp*) for *CREB1* allele length variation. ANZO population data points (triangles) and SM population data points (circles); 100 posterior draws (grey lines); change point posterior distributions (four blue density curves, each representing one chain); and 89% prediction intervals (grey dashed lines). (a) Relationship with population age, showing a higher mean length in recently colonized populations and their Tasmanian source population. Change point posteriors range from ~200 years ago to ~4000 years ago. (b) Relationship with dispersal index (DI). The highest DI corresponds to Tasmania followed by New Zealand and Chatham Islands. Norfolk Island is among the young populations with no outgoing gene flow but long mean *CREB1* length. The change point posterior is centred between the DI values for Chatham Islands and Efate.

genotype). This is because the colonizing flocks were likely a mix of dominant dispersive leaders and submissive individuals as the silvereye is a social species (Kikkawa, 1987). Natural selection could have acted onto this phenotypic variation and associated genotypes. Another mechanism by which the same phenotype could have arisen is through new mutations in each of the colonizations. However, this scenario is unlikely because standing variation is a more relevant mechanism driving parallel evolution at shallow timescales (Hedrick, 2013).

At the broad scale, the most dispersive populations, and those evolutionarily recent colonization events, carry longer allele lengths, compared to more sedentary, and more ancient island populations. This is the opposite pattern to that found in common buzzard (*Buteo buteo*) where non-dispersive individuals carried longer *CREB1* allele lengths (Chakarov et al., 2013). *CREB1* allele lengths have also been shown to correlate with other life-history traits that also depend on circadian and circannual clocks e.g. incubation duration (Bourret & Garant, 2015) and male moult speed (Bazzi et al., 2017). The broad scale patterns, and suggested association with dispersal, were not always reflected within each of the longer- and shorter-allele length groups. For instance, within SM, Gaua and Espiritu Santo displayed the longest lengths, but according to our gene flow analyses, Espiritu Santo was a dispersive population within its grouping but Gaua was not; likewise for Norfolk Island in the ANZO cluster.

While the obvious length variation pattern seen at *CREB1* appears to have biological relevance for dispersal propensity in our study system, a mechanistic resolution of the paradox of the great speciators also requires dispersal shifts to be rapid (Diamond et al., 1976). We were unable to pinpoint when the *CREB1* allele size reduction occurs because of a lack of island populations of intermediate ages. At best we can say that for silvereyes, a shift in dispersal propensity is relatively rapid, taking more than 63–95 generations (South Island New Zealand, 190 years since colonization, generation time of 2–3 years) and <1–1000 generations (Heron Island, 3000–4000 years old) (Clegg et al., 2008).

Variation at a second gene, *CLOCK*, was associated with migratory status of individuals in a partial migrant population, with migrant individuals having longer *CLOCK* allele variants, including long variants absent in non-migrant individuals from Tasmania, Australian mainland and all island populations. Migrant and sedentary individuals did not show genome-wide population structure, suggesting that *CLOCK* could potentially be a gene under divergence. Previous studies in migratory birds focusing on *CLOCK* variation show mixed results. Longer allele lengths have been found to be positively associated with migratory propensity (Peterson et al., 2013), and variation in the phenology of migratory species (Bazzi et al., 2015; Caprioli et al., 2012; Liedvogel et al., 2009), but in some cases allele lengths are negatively correlated with migration date (Ralston et al., 2019) or not correlated at all (Contina et al., 2018; Mueller et al., 2011; Parody-Merino et al., 2019).

CLOCK plays a key role in regulating the circadian oscillator gene complex (Panda et al., 2002), and is associated with variation in the phenology of photoperiodic traits (e.g. migratory behaviour) (Table S1). For example, in migratory equatorial African stonechats annual-cycle timing is associated with *CLOCK* length (Justen et al., 2022). Increasing photoperiod can initiate migration (Assadi & Fraser, 2021; Robart et al., 2018). In silvereyes, photoperiodic stimulation experiments resulted in migratory restlessness being

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triggered only in migratory caged birds but not in resident ones, supporting a genetic link with migratory behaviour (Chan, 1994). Consequently, photoperiod changes could be the migration trigger with the onset of shorter autumnal days. The Tasmanian population is one of the few silvereye migrant populations situated where changes in day length during the non-breeding, winter period is substantially greater than changes experienced further north and closer to the equator. Another location where these conditions are met is New Zealand. In fact, even though records are few, large flocks of silvereyes seem to have migrated from the South to the North Island (Dennison et al., 1987), providing further support that photoperiod changes which are mediated at least partially by genetics, can lead to migration. Some overwater dispersal events might be a direct consequence of off-course migration. For example, the recent sequential colonization sequence of the silvereye from Tasmania to New Zealand and outlying islands was likely initiated by an off-course flock of Tasmanian migrants (Mees, 1969). However, Tasmanian migrants sampled in this study carry long *CLOCK* allele variants that were not recovered in the recently colonized populations. This could because we are not sampling the full allelic diversity of *CLOCK* in recently colonized populations (New Zealand ($n = 6$), Chatham Island ($n = 13$), Norfolk Island ($n = 2$); Table S3), and/or underlying effects of a different set of genes that we have not included in this study on dispersal ability in these populations. The genetic basis of partial migration in Tasmanian silvereyes and whether associated variants are present in recently colonized populations should be further explored in future work using a hypothesis-free genome-wide approach.

DRD4 is one of the most well-studied candidate genes related to exploratory and risk-taking behaviour (Bubac et al., 2020)—both of which could feasibly have links to dispersal propensity. We found a single polymorphism within *DRD4* (SNP83) that showed fixed differences between two young (more dispersive) and seven old (more sedentary) island populations. The Tasmanian population was polymorphic (G and A represented), the recently established populations of Chatham Island and South Island New Zealand were fixed for A, and Southern Melanesian populations fixed for G. SNP83 is located in intron 2 and corresponds to base pair 9423 on the *DRD4* orthologue of the great tit (Fidler et al., 2007). It has not been noted previously as having any phenotypic associations. Previous work assessing *DRD4* variation and personality in the great tit focused on associations between exploratory behaviour and variation at 'SNP830', revealing large effects in certain populations but not in others (Fidler et al., 2007; Korsten et al., 2013; Riyahi et al., 2017); however, SNP830 was not variable in our dataset. Our data add to the evidence that variability in the *DRD4* gene plays a role in a suite of behavioural phenotypes; however, the extent of importance of SNP83 will require screening more individuals and populations in silvereyes and other species.

Even though *NPAS2*, *ACDYAP1* and *SERT* each showed some variability between populations this variability is not related to dispersal ability. Given the limitations of studying a handful of candidate genes for explaining complex behavioural phenotypes, failure to detect associations is not entirely unexpected. In birds, different associations (negative, positive or no correlation) in different populations, species and candidate genes are often reported (Table S1). Furthermore, few studies have considered candidate gene–environment interactions (but see Bourret & Garant, 2015; Liedvogel & Sheldon, 2010; Liedvogel et al., 2009) or methylation patterns that have been found to explain diverse complex behaviours in birds (Saino et al., 2019). The lack of replicability even within the same species could also be a product of sampling in different locations with a low number of individuals leading to a lack of statistical power, choosing different variables and proxies to measure migration and dispersiveness, using different methodologies to analyse data, publication bias or a combination of all of these (Yang et al., 2022). Thus, the lack of association in *NPAS2*, *ADCYAP1* and *SERT* does not rule out their potential role in dispersal behaviour as other factors might be masking their effects.

4.1 | Maintenance of high dispersal propensity in a continental island population

Despite being an old insular form (>200 K split from Australian mainland subspecies Black, 2010), the Tasmanian silvereye (*Z. l. lateralis*) has maintained high dispersal propensity: it is a partial winter migrant to mainland Australia, it was the original source population for the historical sequential colonization of New Zealand and outlying islands (Clegg et al., 2002; Mees, 1969), and as shown here, displays high levels of gene flow with Australian mainland subspecies. It also maintains the longer *CREB1* average allele lengths, and putative Tasmanian migrants caught on the mainland have shown unusually long *CLOCK* mean allele lengths despite genome-wide lack of divergence. The maintenance of high dispersal potential, and its partial migrant status, are most likely explained by its geography and history of connectivity with the mainland. Tasmania has repeatedly been connected to the Australian mainland during glacial periods and it is currently separated by a very shallow sea (Blom & Alsop, 1988). Over 50 islands of varying sizes can be found between the Australian mainland and Tasmania, which can act as migration stopovers, and facilitate connectivity (Belbin et al., 2021).

4.2 | The future of dispersal genomics

A candidate gene approach to understanding the paradox of the great speciators relies on knowledge of those genes in multiple systems. As discussed earlier, the candidate gene approach has many limitations and often shows conflicting results. Alternative hypothesis-free approaches, like genome-wide association studies (GWAS), partially overcome the obstacles imposed by the incomplete understanding of the mechanisms underlying complex behaviours. GWAS requires phenotypes, in this case dispersal propensity, to be characterized at the individual level. Tasmanian migrants were relatively easy to identify because of their different morphological differences with the mainland subspecies. However, assigning a dispersal score to individuals where morphology cannot be used to

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distinguish between dispersers and non-dispersers becomes challenging. Dispersal phenotypes could be assessed via use of individual tracking devices though the sample sizes for these types of studies are often smaller than required for GWAS in particular.

Even though our results suggest that migrants have genetic differences from non-migrant individuals, and that more dispersive populations have different *CREB1* profiles to non-dispersive ones, a more thorough sampling involving a higher number of loci is necessary to explore whether standing genetic variation within a population or de novo mutations can provide the raw material for natural selection to act upon shifting a population to complete sedentariness.

5 | CONCLUSION

In this study we assessed variation in six personality-related candidate genes in silvereye populations to examine whether signatures consistent with a genetic switch can explain rapid shifts in dispersal, leading to reduced gene flow and ultimately divergence in this great speciator. We find strong support for the idea that more dispersive populations carry longer *CREB1* alleles, but length decreases with time and limited isolation, suggesting that selection could be acting against dispersal ability following island colonization. At the individual-level, partial migrants showed longer *CLOCK* alleles than non-partial migrant individuals in a population. Our results suggest that the paradox of the great speciators can be partially understood from a genetic perspective.

AUTHOR CONTRIBUTIONS

Andrea Estandia: Data curation (lead); formal analysis (lead); methodology (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Ashley T. Sendell-Price:** Data curation (supporting); writing – review and editing (supporting). **Graeme Oatley:** Resources (equal). **Fiona Robertson:** Resources (equal). **Dominique Potvin:** Resources (equal); writing – review and editing (supporting). **Melanie Massaro:** Resources (equal); writing – review and editing (supporting). **Bruce C. Robertson:** Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); writing – review and editing (supporting). **Sonya M. Clegg:** Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); writing – original draft (lead); writing – review and editing (lead).

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.63xsj3v6f and supplementary material is available at https://doi.org/10.5281/zenodo.7560902.

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5

Standing genetic variation and *de novo* mutations underlie parallel evolution of island bird phenotypes

Under review

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Standing genetic variation and *de novo* mutations underlie parallel evolution of island bird phenotypes

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Parallel evolution occurs when the same trait evolves in closely related lineages in response to similar ecological contexts and provides some of the best examples of determinism in evolutionary biology. However, from a genetic standpoint, this process can be driven by either new mutations that appear independently in each diverging population or by selection on existing genetic variation common to both lineages. Small-bodied birds, for example, tend to increase in size after they colonise a new island, following what is known as the 'island rule'. Such is the case of the silvereye (*Zosterops lateralis*), a prolific natural coloniser of southwest Pacific islands. Island forms of this bird species increase in body size after they establish, with the pattern and pace of change consistent with directional natural selection and evident even in the most recent colonisations within the last 200 years. The system provides an exceptional opportunity to explore the genomic basis of repeated body size evolution. We sequenced 377 whole genomes from 31 different silvereye populations, which revealed that both mechanisms are at play: in some lineages, new mutations are highly associated with body and bill size, but there are also highly associated polymorphisms present across all populations. Our research sheds light on the genomic basis of repeated body size evolution and emphasises that multiple molecular mechanisms can underlie similar evolutionary trajectories even within a single taxon.

parallel evolution | adaptation | island rule | GWAS | inversion | structural rearrangements | mutations | body size | standing genetic variation | birds
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Introduction

Parallel evolution refers to the independent emergence of similar traits in different lineages that share a common ancestor (Cerca 2023), and illustrates deterministic evolutionary outcomes that are widespread across the Tree of Life (Blount et al. 2018). Natural selection is typically responsible for parallel evolution, as genetic drift is unlikely to produce such similar changes (Arendt and Reznick 2008; Stayton 2008; Losos 2011; McGhee 2011). Selection can act on standing genetic variation to produce the same phenotype (Lai et al. 2019; Therkildsen et al. 2019; Konečná et al. 2021), on different *de novo* mutations in each population (Fulgione et al. 2022; Cerca et al. 2023), or a combination of the two (Ament-Velásquez et al. 2022). However, a faster rate of adaptive change is more often facilitated from standing variation than from mutations as the latter are mostly neutral or mildly deleterious (Hedrick 1983; Barrett and Schluter 2008). For example, the rapid repeated evolution of stickleback low-plated phenotypes is driven by reassembling ancestral variation (Colosimo et al. 2005; Archambeault et al. 2020). Additionally, standing genetic variation can be supplemented with genetic variants that contribute to parallel evolution shared via admixture among recently diverged lineages (Taylor et al. 2020; Montejo-Kovacevich et al. 2022; Cerca et al. 2023; Wang et al. 2023). The same processes can affect single polymorphisms or full blocks in the genome: chromosomal rearrangements, like inversions, can play a role in parallel evolution by keeping sets of adaptive alleles together in strong linkage disequilibrium (Faria et al. 2019; Harringmeyer and Hoekstra 2022). Understanding the relative importance of alternative genetic mechanisms behind parallel evolution has received considerable interest over the last few decades (MacPherson and Nuismer 2017; Westram et al. 2022). Genomic methods now allow us to investigate examples spanning diverse systems, providing empirical data that is necessary to draw generalities.

Islands provide environments where comparable evolutionary pressures can result in the rapid emergence of similar phenotypes (Baeckens and Van Damme 2020). The 'island syndrome' refers to the phenomenon where sets of morphological, physiological, and behavioural traits evolve in a predictable way when organisms colonise islands. The syndrome ultimately results from the exposure to a common suite of biotic conditions on islands; a combination of reduced predation and parasitism, and a shift in the balance of intra- and interspecific competition, features that fundamentally change the selective pressures in predictable and repeatable ways (Clegg and Owens 2002; Meiri et al. 2004; Lomolino 2005). Arguably the best-characterised component of this syndrome is the 'island rule' that describes evolution towards medium body sizes in insular settings; small colonising species evolve larger body sizes and vice versa (Foster 1964; Clegg and Owens 2002; Benítez-López et al. 2021). There is evidence for the island rule across vertebrate taxa, including birds (Benítez-López et al. 2021). Bill size also follows this rule whereby short-billed species show an increase in size (Clegg and Owens 2002). Body and bill size are complex multivariate quantitative traits predicted to be highly polygenic (Yang et al. 2011b; Bosse et al. 2017; Duntsch et al. 2020), but some studies in natural populations show that a few genes of major effect can also impact these traits (Lamichhaney et al. 2015, 2016; vonHoldt et al. 2018; Duntsch et al. 2020).

Members of the silvereye subspecies complex (*Zosterops lateralis*, Aves) have repeatedly colonised islands across the South Pacific from the Australian mainland and Tasmania, with up to 17 morphological subspecies currently recognised (Clements et al. 2021). Insular silvereyes are larger than

mainland ones, and the degree of this shift increases with time since colonisation (Clegg et al. 2002a), a pattern that has been shown to be a consequence of directional selection rather than drift (Clegg and Owens 2002; Clegg et al. 2008). The island populations represent at least four independent colonisations from the Australian mainland: to Southern Melanesia (∼150,000 years ago), Lord Howe Island (∼100,000 years ago), southern Great Barrier Reef Islands, including Heron Island (∼4000 years ago) and a recent historically recorded sequential colonisation sequence from Tasmania to New Zealand and outlying islands (<200 years ago) (Black 2010) (Fig. 1). The populations arising from these independent colonisations are genetically isolated from the original mainland source and other colonisations, though gene flow occurs among Southern Melanesian islands and among recently colonised populations (Estandía et al. 2023). The combination of independent and known colonisation sequences, along with broadly repeated shifts in body and bill size in similar ecological contexts provides an excellent system to explore the genetic mechanisms of parallel evolution. Here, we ask whether *de novo* mutations or standing genetic variation lead to the same phenotype: increased body and bill size in small-bodied species after island colonisation. We find that both are associated with body size variation in Southern Melanesia, whereas only standing genetic variation appears important for repeated patterns in all other populations. This demonstrates empirically that two genetic mechanisms hypothesised to underlie repeated evolution can operate simultaneously in a single system.

Results

The genetic basis of repeated body and bill size evolution.

To describe and quantify the bill and body size shifts in the populations studied here, we summarised univariate traits as principal component scores, resulting in a PC1 for body, and a PC1 for bill that represented size. In both cases 75% of the variation was explained by PC1 and roughly equal trait loadings of univariate traits (Fig. 1; Table S1). We statistically confirmed increases in body and bill size (Fig. S1; Table S2). To examine the genetic basis of repeated evolution, we generated low-to-intermediate coverage (5X) whole-genome sequencing data of 377 silvereyes from 24 islands and sampled across seven Australian States and Territories representative of most of the silvereye range (Table S3). After alignment of the sequenced individuals, we analysed genetic variation in a probabilistic framework accounting for low coverage (ANGSD (Korneliussen et al. 2014)) and obtained 14.3 million SNPs with minor allelic frequencies higher than 5% for differentiation analyses.

Population structure was described by a genomic PCA based on whole-genome data that revealed three major groups (Fig. S2 A). PC1 explained 20.18% of the total variance and separated two clusters: one containing individuals from Southern Melanesia (SM cluster henceforth) and the other containing the remaining individuals (ANZO cluster). PC2 explained 6.8% of the variance and separated the two main archipelagos within SM: New Caledonia and Vanuatu. This population structure aligns with previous analyses using fewer individuals (Estandía et al. 2023). We visualised further substructure within the SM and ANZO clusters (Fig. S2 B-C).

We controlled for the population structure described above by using the eigenvectors that explained most of the variation, and conducted a GWAS that identified multiple loci linked to body size variation suggesting a polygenic basis for the trait. However, given the complicated population structure of our system, we retained those associated variants within the top 50 for which we had evidence of intra-population variation associated with body size, a total of 36 variants. We found that eight were classified as putative *de novo* mutations exclusive to islands in Southern Melanesia (Fig. 2A; Table S4), five of which were part of an association peak on Chromosome 2, which also contained one annotated gene, *GRHPR*, important in metabolic processes (Rumsby and Cregeen 1999). For the top outliers, those individuals with the genotype BB showed a five times increase in median body size relative to those with the AA genotype (Table S5), and the effect was still strong when we only explored Central Vanuatu, where the putative new mutation seems to be restricted to (Fig. 2A). This associated peak emerged again when we conducted GWAS on Southern Melanesia alone (Fig. S3). The effect size of the SNPs across this region was greater than any of the covariates included in the model to capture any population structure, suggesting no signs of conflation (Fig. S4). Other size-associated loci showed a similar effect in direction and magnitude on body size. Among these, were genes involved in calmodulin binding, cytoskeletal motor activity, organisation of the extracellular matrix and other metabolic processes (Table S6).

All of the top associated loci with bill size that were variable within populations represented standing genetic variation (Fig. 2B; Table S7). A number of adjacent variants on Chromosomes 12 and 18 were found in several of the large bill-size populations: islands in Southern Melanesia, Heron Island and Lord Howe Island. Among the annotated genes for bill size, we found gene *LGR6* (Table S8).

We quantified the proportion of phenotypic variance explained by all genome-wide SNPs with GCTA (Yang et al. 2011a). These narrow-sense heritability estimates were high but showed wide confidence intervals (CI) (Table S9). Tarsus length is a good proxy for body size and it is a highly heritable trait based on pedigree data, with a heritability of 0.7 for Heron Island silvereyes (Clegg et al. 2008), a higher value than our SNP-based estimate (h^2=0.5 (95% CI =0.3-0.8)).

Structural variation: a neo-sex chromosome and multiple putative inversions.

To examine the structural variation along the genome, we identified clusters of PCA windows (local PCAs) based on Multidimensional Scaling (MDS) (Fig. 3-4). This method uses common patterns to identify structural variation such as non-recombining haplotype blocks (Li and Ralph 2019; Mérot et al. 2020). We found a large outlier region on Chromosome 4A (Fig. 3A-3B). MDS1 separated two distinct groups that aligned with sex (Fig. 3C), where sex was

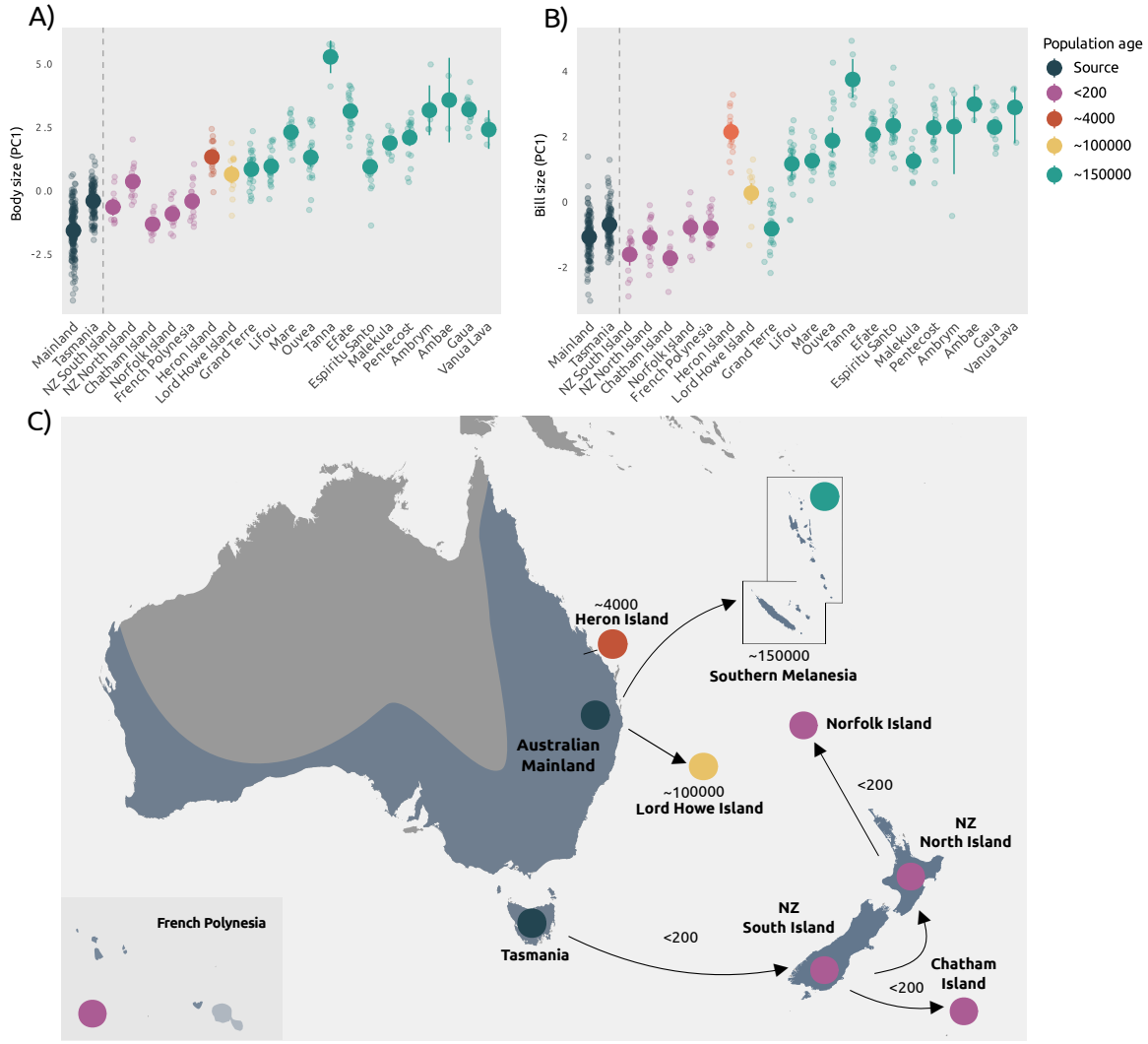


Fig. 1. Morphological divergence in A) body and B) bill size (PC1) among silvereye forms. The source populations are placed left to the vertical dashed line. Colonisations are arranged chronologically: the most recent ones on the left and the most ancient on the right. C) The map shows the silvereye distribution highlighted in blue and the time of colonisation of each island or archipelago. French Polynesia is shown as inset.

independently confirmed by PCR-based sexing of individuals.

To specifically search for putative inversions, we filtered those sections of the genome with at least five adjacent outlier windows, pooled the windows and repeated MDS analysis for each separate region. Several chromosomes showed large outlier regions that formed three discrete equidistant clusters along MDS1. This type of pattern is highly suggestive of a potential inversion with each alternative homokaryotype separated by the intermediate heterokaryotype (Westram et al. 2022) (Fig. 4B). Consistent with the occurrence of inversions, LD was high inside these regions contrasting to the LD along the rest of the chromosome, indicating limited recombination in heterokaryotypes. For the potential inversion on Chromosome 27, the AA karyotype (where A indicates the most common version of the arrangement) was present at high frequencies in all intermediate and old colonisations (Southern Melanesia, Lord Howe Island and Heron Island), and at very low frequency on the mainland. The potential inversion on Chromosome 28 showed a different pattern with the AA karyotype exclusively present in Southern Melanesia. On Chromosome 29, BB was only recovered from individuals in Southern Melanesia, but some individuals from New Caledonia displayed the AA karyotype (Fig. 4). Body size

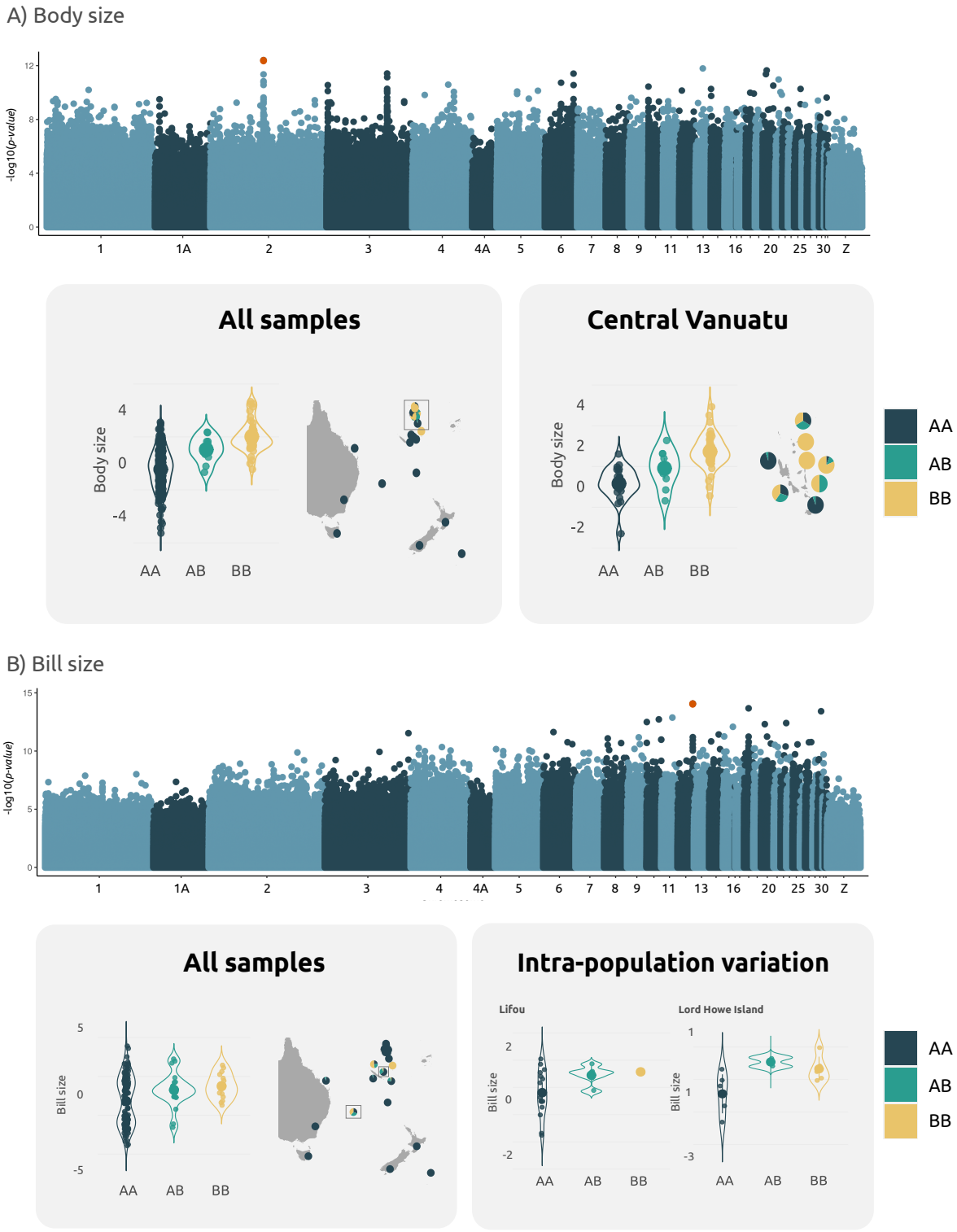


Fig. 2. A) Manhattan plot for body size (PC1) and correlation between allele frequency for the top-ranked SNP and its geographic distribution across all samples (left) and restricted to Central and North Vanuatu, partly where the putative *de novo* mutations occur (right). It is important to note that this set of islands show minimal population structure. B) Manhattan plot for bill size (PC1) and correlation between allele frequency for the top-ranked SNP and its geographic distribution across all samples (left) and across two of the populations where there is intra-population variation associated with body size (right). These two populations are part of separate colonisations.

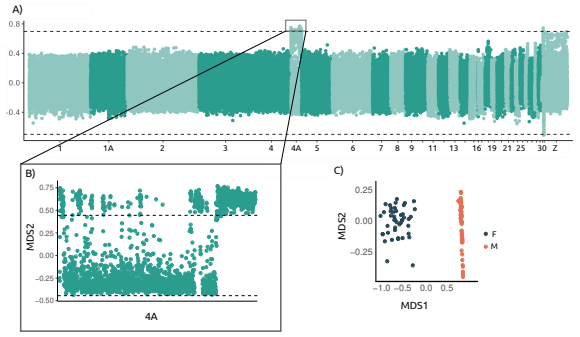


Fig. 3. A) Local PCA results (MDS1) plotted across the genome B) where chromosome 4A shows outlier windows each representing 100 SNPs. C) MDS1 and MDS2 separate by sex in this region.

was not associated with karyotype for Chromosomes 27 and 29 after controlling for population structure. Body size was associated with the AA karyotype in Southern Melanesia, but we could not control for population structure as karyotype and neutral variation structure were entirely coincident. Using our custom annotation, we found genes involved in various metabolic processes on Chromosomes 27 and 28, and lipogenesis, adipocyte differentiation and muscle tissue development on Chromosome 29 (Table S10).

Discussion

Selection on standing genetic variation is a common route to repeated evolution of larger body and bill sizes in island-colonising silvereyes. Size-associated variants were shared across colonisation histories spanning hundreds to over a hundred thousand years. However, putative *de novo* mutations present only in Southern Melanesia silvereye populations were also linked to increased body sizes. This empirical system suggests the joint role of standing genetic variation and *de novo* mutations in producing a repeated island phenotype.

We identified several candidate genes common across all populations. This pattern suggests that variation present in ancestral source populations was represented in multiple, independent colonising flocks, and maintained across sequential island colonisations. Given that population founding can result in loss of genetic variation and the fate of rare alleles can be highly stochastic especially when populations remain small (Whitlock 2000; Sendell-Price et al. 2021), such an outcome might seem unlikely. However, silvereyes colonise in reasonably large flocks (>100 individuals), and they recover rapidly from small population sizes, minimising loss of variation during founding and population establishment (Clegg et al. 2002a; Sendell-Price et al. 2021). Historically colonised silvereye populations and Heron Island silvereyes have experienced rapid trait evolution driven by natural selection (Clegg et al. 2002b, 2008; Sendell-Price et al. 2020), and such rapid evolution is more likely to stem from standing genetic variation than *de novo* mutations (Marques et al. 2019).

However, an association peak harbouring putative *de novo* mutations was present exclusively in Southern Melanesia, the first region to be colonised by silvereyes. These mutations were not fixed across all populations: some of them had fully fixed BB genotypes associated with very large body sizes, while others lacked the minor allele entirely, and many displayed all genotypes. This pattern held even within populations, indicating no bias due to population structure. Variation could be maintained across the archipelagos if different degrees of size are favoured in different populations connected by gene flow as seen across Southern Melanesian silvereye populations (Clegg and Phillimore 2010; Estandía et al. 2023). While larger size of island silvereyes appears generally to be favoured, other species, and indeed other *Zosterops* white-eye species that co-occur, could impose local constraints on size. For example, on Lifou, an island in New Caledonia, three *Zosterops* species occur in sympatry: the relatively small-sized endemic *Z. minutus*, the large endemic *Z. inornatus*, with the silvereye being intermediate in body size between the two (Mees 1969). An alternative but untested possibility is that variants arise from introgression with sympatric *Zosterops* species (Gill 1970). Other mechanisms for maintaining variation, such as negative frequency-dependent selection, are more difficult to reconcile in systems like islands where the usual drivers, e.g. predation risk, are limited (Beauchamp 2021).

Our study was unable to determine the prevalence of *de novo* mutations versus standing genetic variation. Even though we were able to detect new mutations in the oldest colonisation, our sample size was denser for this region than for any of the other of the old-to-intermediate colonisations which might have affected detection of association loci private to these populations. For these reasons, we can only say that in our study system, both routes seem to underlie body size variation, but their relative importance remains an important question to tackle in future studies.

Although linking annotated gene function to phenotypes in non-model organisms is highly speculative, any associations can provide the basis for future investigation. The putative *de novo* association peak on Chromosome 2 associated with body size has a hit for *GRHPR*. This gene encodes the enzyme glyoxylate reductase/hydroxypyruvate reductase, and is involved in key metabolic processes, including serine metabolism for gluconeogenesis and oxalate metabolism (Rumsby and Cregeen 1999). No other previous study has linked body size variation with this gene, yet, avian comparative genomic studies suggest that enzymes involved in glyoxylate metabolic routes might be responsible for diet-related adaptations in birds (Zhang et al. 2014). The function of other genes associated with body size is variable, from bone and muscle development to metabolic processing, and even though their role in body size is feasible, exact mechanisms remain to be found.

LGR6 was one of the genes associated with bill size variation. This gene acts as a mediator for the Wnt/β-catenin signalling pathway, known to be involved in the formation of the avian beak (Bhullar et al. 2015). Interestingly, we did not find significant associations with *ALX1* or *HMGA2*, two

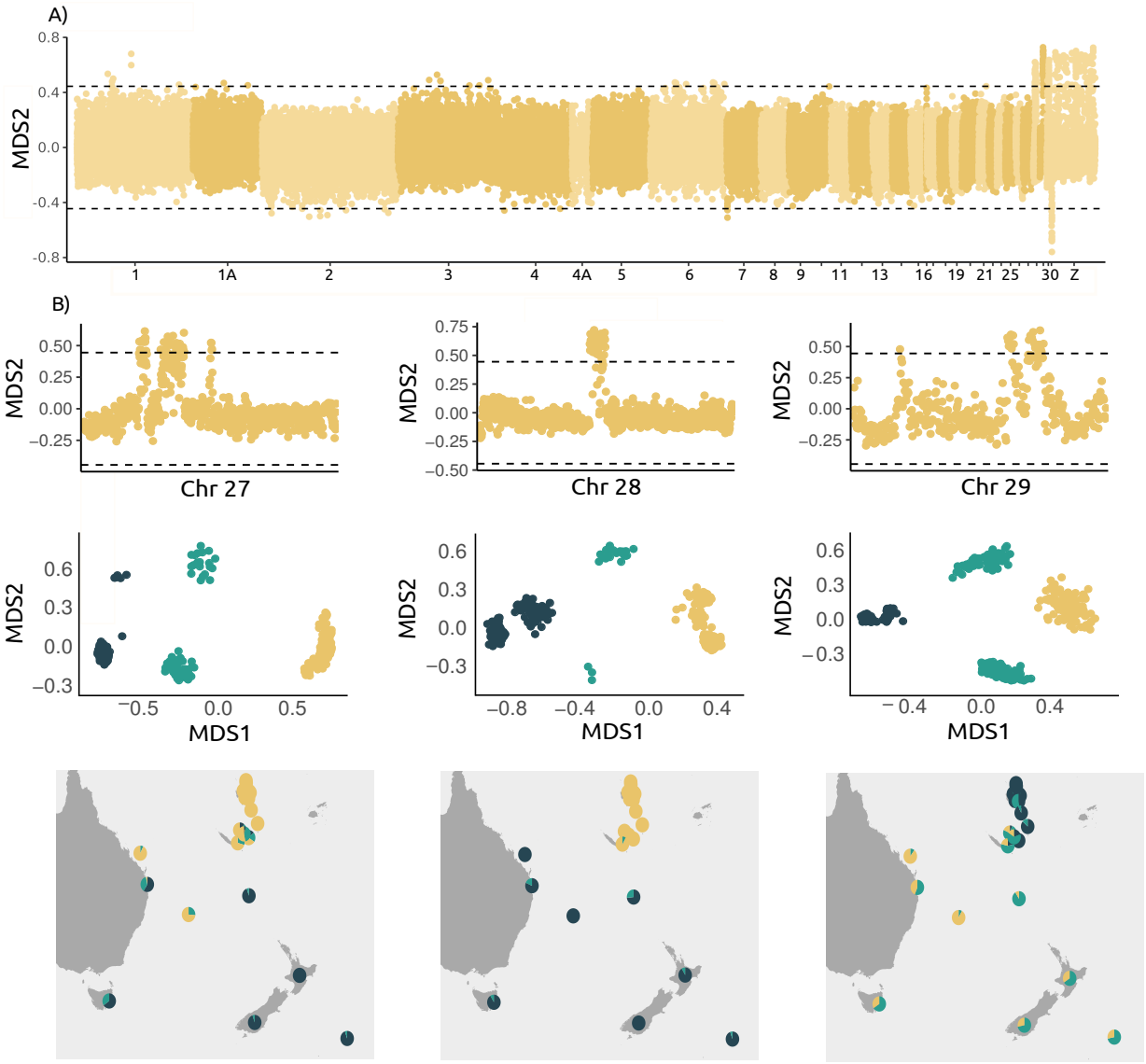


Fig. 4. A) Local PCA results (MDS2) are displayed across the entire genome. B) Outlier regions in chromosomes 27, 28, and 29. Each dot corresponds to 100 SNPs. MDS1 identifies three distinct clusters within the outlier regions, suggesting potential inversions. Chartpies provide information about the relative frequencies of each cluster within different populations.

large-effect genes that have been repeatedly demonstrated to be implicated in bill morphology variation, including in Darwin's finches (Lamichhaney et al. 2015, 2016). However, different genetic architectures are not entirely surprising; even other species of finches show different pathways underlying bill size variation (vonHoldt et al. 2018).

We identified signatures of structural variants, one of which could potentially contribute to body size variation on chromosome 28, but others where such a relationship was clearly absent. Rearrangements on chromosomes 27, 28 and 29 each showed a pattern consistent with an inversion: three different clusters separated by MDS1 and high LD within the putative inversion compared with the rest of each chromosome (Mérot et al. 2020; Westram et al. 2022). Our conservative size estimates of the putative inversions observed here (approximately 200kb for those on Chromosomes 28 and 29 and 650 kb for Chromosome 27) place them in the range of inversion sizes known to be most frequently associated with adaptive phenotypes (100kb to several Mb) (Faria et al. 2019). Reduced recombination in the heterokaryotypes preserves linkage among alleles despite mating between alternative homokaryotypes, maintaining the adaptive benefit of the haplotype blocks (Faria et al. 2019; Westram et al. 2022). For example, marine snails (*Littorina saxatilis*) and seaweed flies (*Coelopa frigida*) show inverted regions linked to adaptive phenotypes (Morales et al. 2019; Koch et al. 2021); the latter has an inversion under balancing selection that works as a supergene for body size. The karyotype distribution of

the putative inversion identified on Chromosome 28 was associated with body size but was conflated with geographic population structure. The function of genes inside this region varied widely, some of them are involved in immunity, others in cholesterol metabolism and some are even directly linked to fitness proxies in birds (Krist 2011), like egg quality (Gao et al. 2021). However, it is unclear whether they contributed to the evolution of large body sizes in Southern Melanesia. To further study the role of structural variants we could generate long-read sequencing and recombination maps that help to confirm that they are indeed inversions and to determine breakpoint locations (Mérot et al. 2020; Akopyan et al. 2022).

The three putative inversions displayed karyotype distribution patterns that suggested varying evolutionary histories. The inversion on Chromosome 28 could have emerged after colonisation from the Australian mainland and could have been subsequently fixed in the majority of Southern Melanesian populations. Aside from sampling considerations, alternative scenarios are more complex, requiring different combinations of karyotype loss and maintenance that seem less likely. For putative inversions on both Chromosomes 27 and 29, the maintenance of the heterokaryotype and alternative homokaryotype in many populations suggest they could represent ancient inversions under balancing selection. Such polymorphism can be maintained when the inversion provides a fitness advantage against different selective pressures: if there are epistatic interactions among alleles at different loci (Charlesworth and Charlesworth 1973), if there is associative overdominance (Ohta 1971), or if a locus that is under balancing selection is located within the inversion (Faria et al. 2019). Similar to alternative processes that could explain SNP variation, karyotypes could appear to be balanced if there is introgression resulting from gene flow among different sympatric *Zosterops* species occurring across Southern Melanesia, on Norfolk Island and prior to 20th-century extinction, also on Lord Howe Island, but not Heron Island or New Zealand.

A sizeable structural variant detected via local PCA and visualised by MDS was on chromosome 4A, coinciding with a known neo-sex chromosome in the Zosteropidae (Leroy et al. 2021), formed via fusion between an autosome and a sex chromosome approximately 24 Ma in the Sylvioidea superfamily to which the Zosteropidae belong (Pala et al. 2012; Sigeman et al. 2020). Neo-sex chromosomes can play a role in speciation as they can contain loci for traits that contribute to reproductive isolation (e.g. male courtship display behavioural traits (Kitano et al. 2009)). However, the role of this neo-sex chromosome in speciation within the Sylvioidea, including in the Zosteropidae, is unclear (Pala et al. 2012).

The study of non-model systems in the wild has inherent practical and bioinformatic limitations. Like any empirical study, we had to balance sample size and genomic coverage. GWAS usually relies on a high number of whole genomes with attached individual data. Capturing and taking samples from the birds used in this study covering a vast geographical distribution was a tremendous effort that spanned decades, and sequencing hundreds of genomes remains relatively expensive. Additionally, body and bill sizes are complex multivariate phenotypes that are highly polygenic (Duntsch et al. 2020) and correlate with several other traits (e.g. Estandía et al. 2021) that we did not investigate. Lastly, identifying the causal genes is a major challenge and often needs extra steps following GWAS to confirm the causality of the candidate loci, for example by doing knock-outs (Bengston et al. 2018), which is not possible in wild birds.

Despite these caveats and limitations, work on non-model systems is essential to gain a broad perspective on the different genetic routes that lead to similar phenotypes. The application of genomic methods to the silvereye system, which encompasses a wide range of island colonisation times and repeated evolution of body and bill size, provides a rare example of the role of standing genetic variation on both short and longer timescales of colonisation, with putative *de novo* mutations contributing only to the oldest island populations.

Methods

Sample collection.

We sampled 377 silvereyes (*Z. lateralis*) from 31 islands or sites on the Australian mainland between 1997 and 2017 (Table S3). Birds were caught in the wild using mist nets or traps and we collected 20–40 µl of blood from the brachial wing vein and stored it in 1 ml of Queen's lysis buffer (Seutin et al. 1991). We took a suite of morphological measurements, including the wing length using a metal ruler to determine the maximum flattened chord of the longest primary feather, and the length of the central tail feathers from the base to the tip using dividers. We used dial calipers (accuracy ±0.1 mm) to measure metatarsal length, head length from the rear of the skull to the bill's tip. Additionally, we took various bill measurements using dial calipers, including mandible length, width, and depth at the posterior nostril opening, and mandible length, width, and depth at the anterior nostril opening. Finally, we measured the body weight. Sex cannot be determined in silvereyes with morphological measurements. We selected a random subset of individuals and sexed them using molecular methods described in the section below. All sampling was non-destructive, and we released the birds at point of capture. Complete sampling information is provided in Table S3.

Construction of a pseudochromosome assembly.

We used the Chromosembler tool available in Satsuma2 (Grabherr et al. 2010) to order and orient scaffolds of the *Z. l. melanops* genome assembly (GCA_001281735.1) (Cornetti et al. 2015) into 33 pseudo-chromosomes according to synteny with the Vertebrate Genome Project's Zebra finch (*Taeniopygia guttata*) genome assembly (GCA_003957565.1) (Rhie et al. 2021).

To reconstruct a transcriptome that we could use to generate hints for gene prediction, we used available RNA-seq data for *Zosterops borbonicus* (Leroy et al. 2021). We filtered out low-quality sequence reads (phred quality score < 20) and adaptor sequences using TrimGalore (Martin 2011) and

mapped them onto our pseudo-chromosome assembly using HISAT2 (Kim et al. 2015). We used Trinity (Grabherr et al. 2011) to assemble the transcriptome.

Assembly annotation.

We annotated the resulting pseudo-chromosome assembly using a multifaceted approach of repeat identification and *ab initio* gene prediction using AUGUSTUS (Stanke et al. 2006). We incorporated hints containing extrinsic evidence about the location and structure of three types of genomic features: repetitive regions, short-read transcriptome sequences, and single-copy orthologs. To obtain our hints, we first identified and masked repetitive and low-complexity DNA sequences using RepeatMasker (Smit et al. 2013) with the chicken (*Gallus gallus*) database of repeats. Second, we used BLAT (Kent 2002) to incorporate RNA-seq information as hints. Third, we used single-copy genes in the Aves lineage group odb10 to detect conserved orthologous genes with BUSCO (Manni et al. 2021). We then used BLASTx (Camacho et al. 2009) in the AUGUSTUS output to obtain functional information. More information about specific parameters used, including cut-offs, can be found at https://github.com/andreaestandia/1.0_island_rule_GWAS/tree/main/pipeline/9_annotation

DNA extraction, molecular sexing, library preparation, and sequencing.

We extracted DNA from blood samples using a standard Phenol-chloroform protocol (Seutin et al. 1991). We added 100 uL of blood stored in Queen's buffer to a microcentrifuge tube containing 250 uL of DIGSOL extraction buffer (0.02 M EDTA, 0.05 M Tris-HCl (pH 8.0), 0.4 M NaCl, 0.5% sodium dodecyl sulphate (SDS)) and 10uL of Proteinase K (20mg/mL). Samples were incubated at 55C overnight. Following incubation, we added 250 uL of phenol:chloroform:isoamyl alcohol (25:24:1) and gently mixed the samples for 10 minutes. We then centrifuged the samples at 10,000 rpm for 10 minutes. We transferred the aqueous layer to a new microcentrifuge tube and repeated the previous step by adding the phenol mixture, mixing and centrifuging it. We recovered a new aqueous phase, transferred it to a new tube, added 250uL of chloroform:isolamyl alcohol (24:1), mixed and centrifuged the samples as in the previous steps. We precipitated the DNA by adding 2 volumes of ice-cold 100% ethanol, 1 volume of 2.5M ammonium acetate and 2 uL of glycogen, leaving it overnight at -20C. The next morning we centrifuged the tubes for 10 min at 15,000 rpm at 4C and removed the supernatant. We rinsed the precipitate with 500 uL of ice-cold 70% ethanol and centrifuged the tubes as in the previous step. We removed the supernantant and the left the precipitated DNA to dry at room temperature. Once dried, the DNA was resuspended in 50 uL of TE (TrisEDTA) buffer (0.01 M Tris-HCL (pH 8.0), 0.0001 M EDTA). We quantified the DNA concentration with a Qubit 2.0. DNA extracts were sent to Novogene UK for library preparation and whole genome sequencing at 5X on the Illumina Novaseq 6000 platform (Illumina, San Diego) using paired-end 150bp sequencing reads.

Quality control and genotype calling.

We trimmed reads to remove adapter content and base calls of low quality using fastp (Chen et al. 2018) with default settings and 10 bp trimmed from the start of each read. We aligned cleaned reads of each individual to our pseudo-chromosome assembly using the Burrows-Wheeler Aligner (BWA)-mem algorithm (Li and Durbin 2010). Genotype calling can result in bias when using low-coverage sequencing (Johnson and Slatkin 2007). Given the moderately low sequencing depth of our dataset (5X) downstream analyses were based on genotype probabilities rather than directly-called genotypes. We calculated genotype probabilities in ANGSD (Korneliussen et al. 2014) using the samtools model and keeping SNPs with a minimum allele frequency (MAF) of 0.05. All analyses described below were run considering only biallelic SNPs with a mapping quality score 20. We filtered the bam files by excluding SNPs that showed significant deviation from Hardy-Weinberg equilibrium, recovering a total of 14,330,920 SNPs.

Statistical tests of repeated increase in body and bill size.

Body size is most often represented by multivariate measures, such as Principal Component Analysis (PCA), that account for the covariation among characters and summarise most of the variation in the first two axes, the first representing body size and the second body shape (Rising and Somers 1989). We conducted a PCA that included all morphological measurements that represent body size: wing length, tail length, tarsus length, and head length; and separately bill size: bill length to posterior nostril opening, bill width, and depth at anterior nostril opening. We tested whether colonisations resulted in changes in body size and bill size relative to the mainland using *brms* (Bürkner 2017). Model 1 included PC1 of body size as the response variable and Model 2 PC1 included bill size. Colonisation time was set as a categorical population-level effect with a weak prior in the form of a normal distribution centered in 0 with a standard deviation of 10. This prior gives equal support to the idea that body size can remain unchanged, increase or decrease after colonisation. We controlled for population by setting it as a group-level effect. We used a Gaussian family and the default link function. We ran four chains each with 5000 iterations which included a warm-up of 1000.

The genetic basis of parallel evolution.

To test whether *de novo* mutations or ancestral standing genetic variation led to similar phenotypes, we did a Genome-wide Association Study (GWAS) under the idea that if the associated variants are common to all populations then they are likely due to standing genetic variation but if they are present in a specific population or specific lineage, they may represent *de novo* mutations that have accumulated since the lineage split. This framework assumes no potential gene flow among colonisations, which is supported by genome-wide data (Estandía et al. 2023).

Genome-wide Association Study.

We conducted GWAS on the first two PCs of body size (body size PC1 and body size PC2) and bill size (bill size PC1 and bill size PC2). We ran a generalised linear model implemented in PLINK (Purcell et al. 2007) and controlled for population structure by generating a covariance matrix with PCAngsd (Meisner and Albrechtsen 2018) and decomposing it by using a scaling transformation. We used the first 8 eigenvectors that accounted for most of the variation and adding more components accounted for less than 1% of the variation and did not split populations further. The two main clusters (ANZO and SM) have diverged for a long time, and many of the associated variants could have arisen due to population substructure not represented by the top eigenvectors and phenotypes that did not fully overlap between the ANZO and SM groups (but see Fig. S5). To check that our results held on each subset, we repeated the GWAS on each subgroup while accounting for their own population substructure (Fig S2 B-C). For the top 50 associated variants, we excluded samples with genotype likelihoods lower or equal to 0.33, which indicates that all genotypes have the same probability of being the true genotype. We classified putative *de novo* mutations as those only present in a single colonisation. For example, if an associated variant is only present on Heron Island, it would be classified as a new mutation. However, if the associated variant is present in various populations from separate colonisations, we classified them as arising due to standing genetic variation. This is because it is unlikely that the same new mutation would arise more than once in separate lineages stemming from the same source. Given that our system shows a complicated population structure, we decided to be conservative and further filtered associated variants by keeping those that showed at least one population where there was evidence of intra-population variability associated with body size. We did this by conducting an ANOVA between genotype and body size in each population separately. We also explored the frequency of genotypes and plotted it on maps to explore geographic structuring. We used our custom annotation to find the regions where the outliers were located by selecting all annotated coding regions within 5000 bp upstream and downstream from the associated locus. To confirm that our annotation was robust, we pulled the same number of bp from the reference genome and performed a BLAST search against all organisms. To estimate the proportion of phenotypic variance explained by all genome-wide SNPs while correcting for LD bias we used GCTA (Yang et al. 2011a). We created four quartiles of LD scores and used each individual SNP's LD score for binning as recommended by Evans et al. 2018. We also controlled for population structure using the principal components arising from the PCAngsd analysis. We computed the h^2-SNP 95% CI as +- 1.96 * SE.

Detection of structural variants.

To detect structural variants, we conducted a localised PCA of genetic variation based on the methodology outlined in Li and Ralph (2019). We used PCAngsd and the R package *lostruct* to identify non-recombining haploblocks across the genome. Structural variants like inversions are characterised by high levels of linkage disequilibrium because recombination is often to be negligible in heterokaryotes (Kirkpatrick 2010). For this reason, we used the unpruned SNP dataset containing 14 million SNPs. First, we split each chromosome into non-overlapping windows containing 100 SNPs each, ran PCAngsd and obtained a covariance matrix for each window. We then descomposed each matrix by using scaling transformation and generated a pairwise distance matrix based on PC1 and PC2 for each window. Finally, we mapped similarity using MDS of up to 20 axes. We defined outlier regions as windows with a coefficient of correlation above four standard deviations from the mean. Adjacent outliers with less than 10 windows between them were pooled, and clusters with less than 5 windows were not considered following Mérot et al. (2021). A typical signature of a polymorphic inversion is three groups of individuals appearing on a PCA: the two homokaryotypes for the alternative arrangements and, as an intermediate group, the heterokaryotypes (Mérot et al. 2020). All clusters of outlier windows were examined by MDS as single blocks. We then used K-means clustering to identify putative groups of haplotypes. We explored LD patterns in heterokaryotype versus homokaryotype individuals for the chromosomes where we found outlier regions. We calculated pairwise r^2 values with ngsLD based on genotype likelihoods obtained by ANGSD and created a heatmap where we could visually explore LD levels. We built a model in *brms* to test whether karyotype identity was associated with body size after controlling for population structure. Karyotype was set as a categorical population-level effect and body size as the explanatory variable. Population was set as a group-level effect. We used a Gaussian family and the default link function. We ran four chains each with 5000 iterations which included a warm-up of 1000. We refer to karyotypes AA, AB, and BB where A represents the most frequent variant, whether inverted or not since we do not have information about this.

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Author contributions

SMC and AE formulated the idea and SMC supervised the project. SMC, BCR, and ATSP conducted fieldwork. AE, ATSP and BCR conducted laboratory work. AE conducted the genetic and statistical analyses with input from ATSP. AE drafted the manuscript with input from SMC and all authors provided comments.

Data and code availability

Code to reproduce all analyses is available at https://github.com/andreaestandia/1.0_island_rule_GWAS

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6

General discussion

How do species come to be? It is easy to forget how young evolutionary biology is as a field. However, in a bit more than 150 years, our knowledge of how species arise, how they disappear and everything in between, has significantly advanced. In this thesis, I explored how islands drive repeated patterns of evolution and how some of these traits may catalyse diversification.

Islands have many things in common that make them comparable and simple study settings: they provide isolated environments and they have a limited number of species (Losos & Ricklefs, 2009). Because of their common selective pressures, such as reduced predation and parasites, increased intraspecific competition, and decreased interspecific competition, many traits evolve in a relatively predictable way (Baeckens & Van Damme, 2020). In passerines, we see an increase in body size and reduced dispersal ability, among others (Jezierski et al., 2023). On one hand, the repeated evolution of increased body size leads to similar phenotypes evolving on each of the colonised islands. On the other, reduced dispersal leads to little genetic exchange among populations which allows them to evolve independently. An interesting case is that of the great speciators, birds present on many islands with multiple subspecies (Mayr & Diamond, 2001). Reduced dispersal propensity might be favoured in great speciators following island colonisation due to the costs of maintaining high dispersal capacity. This leads to the isolation of populations and local adaptation to their environment, which can hugely vary from island to island, generating a great

morphological diversity (Diamond et al., 1976). This idea fits well with the taxon cycle, the hypothesis that insular taxa evolve in colonisation cycles whereby species expand, differentiate and tend to extinction or undergo further waves of expansion (Wilson, 1961).

When I first started my thesis I decided to read the core piece of work that discusses great speciators: *Birds of Northern Melanesia*, by Mayr and Diamond (2001). I very much enjoyed diving into this book filled with ideas that had been simmering for nearly three decades since their expedition to the South Pacific. I decided that the first part of my DPhil should focus on understanding what great speciators are and quickly I concluded that this term might simply refer to an artificial grouping of birds with an appealing name. Mayr and Diamond probably did not intend to create this discrete group that was later used by researchers seeking to gain attention for their papers. This led to a narrowing of focus on certain birds, rather than on the processes that drive diversification on islands. The scientific community indeed thought there was something special about Northern Melanesia, but never expanded this idea to other island systems and with time, the meaning of the term changed: it started being about species with many subspecies and now it is used loosely, sometimes applied to higher taxonomic levels like families and confused with rapid diversification. After reading extensively about this topic, I wondered whether great speciators (in the original sense) lay at the end of a continuum and indeed had some common traits or whether the particular island-rich region of Northern Melanesia led to their high diversity.

To explore these questions, in my second chapter I curated a global dataset of island bird subspecies and investigated the drivers of incipient speciation on islands. I introduced a quantitative metric, the Great Speciator Index, that revealed that great speciators are the end of a continuous distribution. Many of the species at the high end were mentioned in the original list for Northern Melanesia but birds from other regions also scored high, such as the bananaquit and the yellow warbler from the Americas. One of the most interesting results was the phylogenetic signal was low, suggesting that a great speciator pattern may arise from shared environmental and geographical characteristics rather than a common evolutionary history. Indeed, spatial effects seem to be particularly influential in driving the 'great speciator' pattern, more so than habitat heterogeneity or species traits, which have weaker but predictable effects. Future work includes building a spatially explicit model that incorporates key parameters commonly used in island biogeography

(dispersal, divergence and extinction) and then exploring whether this model fits observations taken from the real world.

Dispersal ability has been proposed to be a key trait in the emergence of great speciators but its role is not clear: some ideas do not involve dispersal (e.g. if the great speciator pattern arises due to changes in sea levels and vicariance), others involve short-distance colonisation, and others propose that great speciators initially have a high dispersal ability but strong selection acts against it following island colonisation (Diamond et al., 1976). Although dispersal is key to understanding great speciators, it is difficult to define as it is a complex trait determined by behaviour, physiology and morphology (Saastamoinen et al., 2018). Although I had individual data for a proxy of dispersal, the Hand-Wing Index (HWI), this approach has proven to be far from ideal. This is because the HWI does not accurately reflect dispersal abilities if there are behavioural adaptations involved. For example, some highly mobile seabirds can show a low HWI but, at the same time, cover extensive areas by doing dynamic soaring whereby the wing can be fully extended without any muscle effort (Pennycuick & Lighthill, 1982). Similarly, swallows are adapted for in-flight foraging, requiring a specific wing shape that results in a high HWI, despite their tendency to not disperse extensively (Sheard et al., 2020). Nevertheless, we found a negative effect of HWI which together with the fact that higher GSI tend to have smaller ranges, and well-separated islands, all point towards lower dispersal ability being an important factor. Additionally, we found that great speciators are small-bodied and tend to have higher diversification rates, aligning with the idea that the processes leading to subspecies and species diversification are interconnected. This chapter introduces a quantitative approach to measure early diversification, challenges categorical distinctions, and reveals that various factors contribute to early population divergence in island bird species.

My third chapter introduced the silvereye for the first time and aimed to reconstruct its evolutionary history. Understanding how silvereyes originate and spread is essential to explore other questions because assumptions rely on an accurate understanding of their evolutionary history. In this chapter, I discovered that the main silvereye lineages have been isolated for a long time with no signs of gene flow, which makes it a good system to explore the evolutionary consequences of island colonisation. Silvereyes have radiated rapidly in just 1.5 million years, giving rise to mul-

tiple subspecies across a wide distribution. On islands, some of these subspecies seem to have been almost entirely isolated (e.g. Lord Howe Island) while others come into contact regularly, such as subspecies occurring on nearby islands in Vanuatu. On the continent, subspecies are highly connected via longitudinal dispersal overcoming major biogeographical barriers and latitudinal partial migration, which also connects the mainland with Tasmania, a continental island. In the phylogeny, uncertainty at some nodes arose either due to signatures of potential gene flow or incomplete lineage sorting due to the recent colonisation of some populations, in particular the sequential colonisation of New Zealand, Chatham Islands and Norfolk Island. Even though this species remains a good system to explore island evolution, this work also provides evidence of the limitations it has. For example, in Chapter 5 I assumed that colonisations emerged from the Australian mainland, but in this chapter, I discuss the (small) possibility that they originated on Pacific islands, colonised the mainland and then recolonised other Pacific islands. This chapter presents the evolutionary history of the silvereye, which will serve as a backbone for future silvereye researchers.

The last two chapters focus on two traits that evolve repeatedly following island colonisation: dispersal ability and body size.

A common consequence of island colonisation is a reduction of dispersal ability as a result of direct negative selection against dispersive individuals (Waters et al., 2020). Reduced dispersal often leads to isolated populations if there is not a regular influx of immigrants, and can ultimately catalyse diversification. As mentioned earlier, dispersal is a complex phenotype (Saastamoinen et al., 2018). As such, evolutionary changes in dispersal ability occur in the underlying traits that have a heritable component and the pace of the change is influenced by the genetic architecture and variance of the trait. The heritability of dispersal varies across species, but it can be as high as h^2=0.95 in Western bluebirds (*Sialia mexicana*), and be underlied by a single gene (Niitepõld & Saastamoinen, 2017), a cluster of linked genes or, most frequently, have a polygenic basis (Mackay et al., 2009). In this chapter, I sequenced six genes associated with dispersal, personality traits and migration to explore whether repeated changes in these genes led to the evolution of sedentariness, assuming that changes would be more likely to arise due to selection on standing variation (Marques et al., 2019). Additionally, I tested whether outgoing gene flow (as a proxy of dispersal)

and population age were associated with genetic variation. We found a decrease in allele length at *CREB1* that did not coincide with neutral genetic structure but was associated with population age, suggesting selection might be operating at this gene. All populations colonised at least 4,000 years ago showed shorter *CREB1* alleles. These results hint at a genetic basis for dispersal, but by using a few loci we miss most of the genomic changes that could reveal the contribution of other loci and the relative relevance of *CREB1*. Although a candidate gene approach is valid and has been used in multiple studies, it is far from ideal as the effect of the changes in allele frequency cannot be quantified. However, we can say with confidence that there are genetic changes associated with dispersal and population age which helps to understand the diversification of the silvereye. Interestingly, I found that, within the Tasmanian population, there was a wide variance at the *CLOCK* gene and that migrant individuals had longer alleles than non-migrants. Partial migration is fundamental to understanding population founding because migratory species can expand their range by colonising new areas accidentally. For example, a migratory flock of silvereyes led to the colonisation of the South Island of New Zealand and subsequently spread to the North Island, Chatham Islands and Norfolk Island (Mees, 1969). Partial migration is likely to be heavily influenced by the environment (Pulido, 2011), but variation at *CLOCK* suggests there might be a genetic component. Although a genomic approach is fundamental to explore these questions, future research should take an integrated approach that also incorporates the role of behaviour and environmental effects as partial migration can be a highly plastic trait, with a clear genetic basis but low heritability. Exploring factors like the role of social dynamics, spatial variation in migration, and potential correlations with climate conditions can provide a comprehensive understanding of the drivers of partial migration crucial for understanding accidental island colonisation, population differentiation and ultimately the emergence of great speciators.

Another trait that evolves predictably after island colonisation is body size, a phenomenon known as the 'island rule'. Silvereyes show an increase in body size in all of the colonisations, with the oldest colonisation having body sizes four times larger than the mainland, but even in the most recent colonisations we see an increase in body size or individual traits, such as in French Polynesia, a human-mediated introduction in the 1900s (Sendell-Price et al., 2020). Repeated evolution can be driven by new mutations, but these arise slowly. A more common route towards

the evolution of similar phenotypes in recently diverged lineages is selection on existing variation. (Marques et al., 2019). These same processes might affect blocks of the genome that are inherited together due to low recombination, such as inversions (Mérot et al., 2020). In Chapter 5, I explored these questions and sequenced almost 400 whole-genomes with associated individual phenotypes representing body size summarised by the first two axes that explain most variation (PC1 and PC2). I did the same with bill size and conducted genome-wide association studies to explore the genetic basis of body and bill size. I assumed that if the associated loci are present across all populations, they likely play a role in body or bill size evolution via selection on that variation, but if they are only in one colonisation it might be underlied by a new mutation. Body size is generally a polygenic trait (Duntsch et al., 2020) and, as expected, many loci were associated with body size variation, but there was an association peak that was only present in populations from North and Central Vanuatu. We verified that the complicated population structure of the system was not biassing our results and concluded this might be a putative new mutation. Our findings emphasise that even at shallow divergences, new mutations can contribute to parallel evolution.

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A

The role of linkage in mimicking "magic traits"

doi:10.1111/evo.14102

Digest: The role of linkage in mimicking "magic traits"

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Can divergence in a mating trait increase local adaption by increasing ecological divergence? Servedio and Bürger propose that "pseudomagic traits," tightly linked complexes consisting of an ecological locus under divergent selection and a locus acting as a mating cue, can effectively mimic pleiotropy. Such pseudomagic traits can form even when linkage between ecological and mating loci is limited.

One scenario for ecological speciation that has recently drawn attention involves so-called "magic traits." A trait should be considered a magic trait when it is both under divergent selection and causes nonrandom mating. Such magic traits are thought to facilitate speciation in the presence of gene flow (Gavrilets 2004) and have been identified in different groups of organisms including animals (e.g., Derryberry et al. 2018) and plants (Schlüter 2018). For example, in ovenbirds (Aves: Furnariidae), traits such as body size and bill shape, which are subject to strong ecological selection, have been found to indirectly influence signal production (i.e., song), which acts as a mating cue (Derryberry et al. 2018). Under divergence with gene flow, magic traits are expected to form a localized peak of divergence (Fig. 1A). However, if recombination is low, a locus under divergent selection and a locus acting as a mating cue that are physically linked can mimic this singular peak of divergence (Fig. 1B), and nonmagic genes may therefore function as if they were a magic trait (Servedio et al. 2011).

In this issue, Servedio and Bürger (2020) gather evidence for the latter scenario, which they term "pseudomagic traits," and further assess whether divergence in both a mating trait and a tightly linked ecological trait can enhance local adaptation. Although it is widely accepted that an ecological trait can subsequently cause divergence in a mating trait, Servedio and Bürger (2020) ask whether the direction of causality can be flipped and whether linkage at the mating trait can cause divergence at the ecological locus. They investigate two logically constructed population genetics models: a two-island model and a continent-island model. These models consist of haploid populations in secondary contact under nonrandom mating, examining both symmetrical and asymmetrical migration. Previous papers hypothesized that the initial strength of linkage disequilibrium (LD) would be fundamental in determining whether or not a pseudomagic trait can mimic a magic trait upon secondary contact. Thus, their models evaluated the importance of the initial LD strength with a population starting in full LD and the same population starting in complete linkage equilibrium.

Servedio and Bürger (2020) find that pseudomagic traits can indeed mimic magic traits, with the presence of a mating trait helping the ecological locus to reach higher levels of divergence, which can ultimately enhance local adaptation. These results suggest that two mechanisms can play an important role in evolutionary divergence: (1) pleiotropy between the mating and the ecological loci (magic traits) and (2) linkage between them (pseudomagic traits). They also explore how tightly linked mating and the ecological loci need to be to produce pseudomagic traits. They find that even loosely linked mating and ecological loci can have a strong effect on evolutionary divergence and enhance local adaptation.

Servedio and Bürger (2020) challenge the singularity of true magic traits during evolutionary divergence, providing an alternative mechanism through which reproductive barriers can rapidly arise. Although theoretical studies such as this one have

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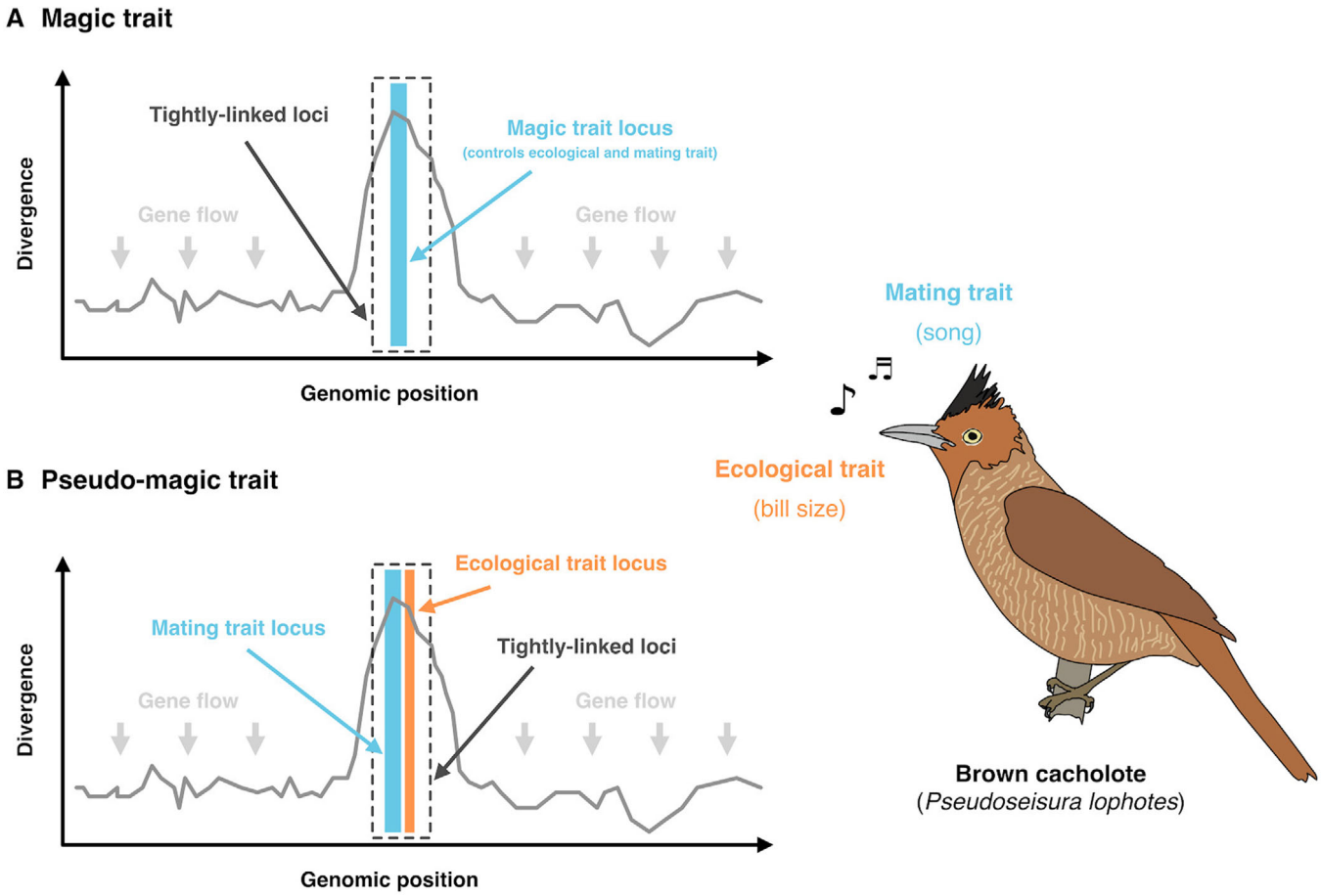


Figure 1. **Illustration showing how magic traits and pseudomagic traits can produce regions of elevated divergence despite gene flow. (A) Selection at magic trait loci results in a region of elevated divergence containing the locus that controls both the ecological trait (bill size) and mating trait in addition to neighboring tightly linked loci. (B) Two separate loci—a mating trait (song) locus and an ecological trait locus which are physically linked result in a region of elevated divergence comparable to selection operating at a magic trait locus. In ovenbirds (Aves: Furnariidae), coupling between an ecological trait (bill size) and a mating cue (song) has been identified (Derryberry et al. 2018)—this likely represents an example of a magic trait.**

advanced our understanding of the role of assortative mating in speciation, further studies need to include an empirical approach and expand the range of scenarios explored (e.g., sympatry).

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B

Supplemental Material Chapter 2

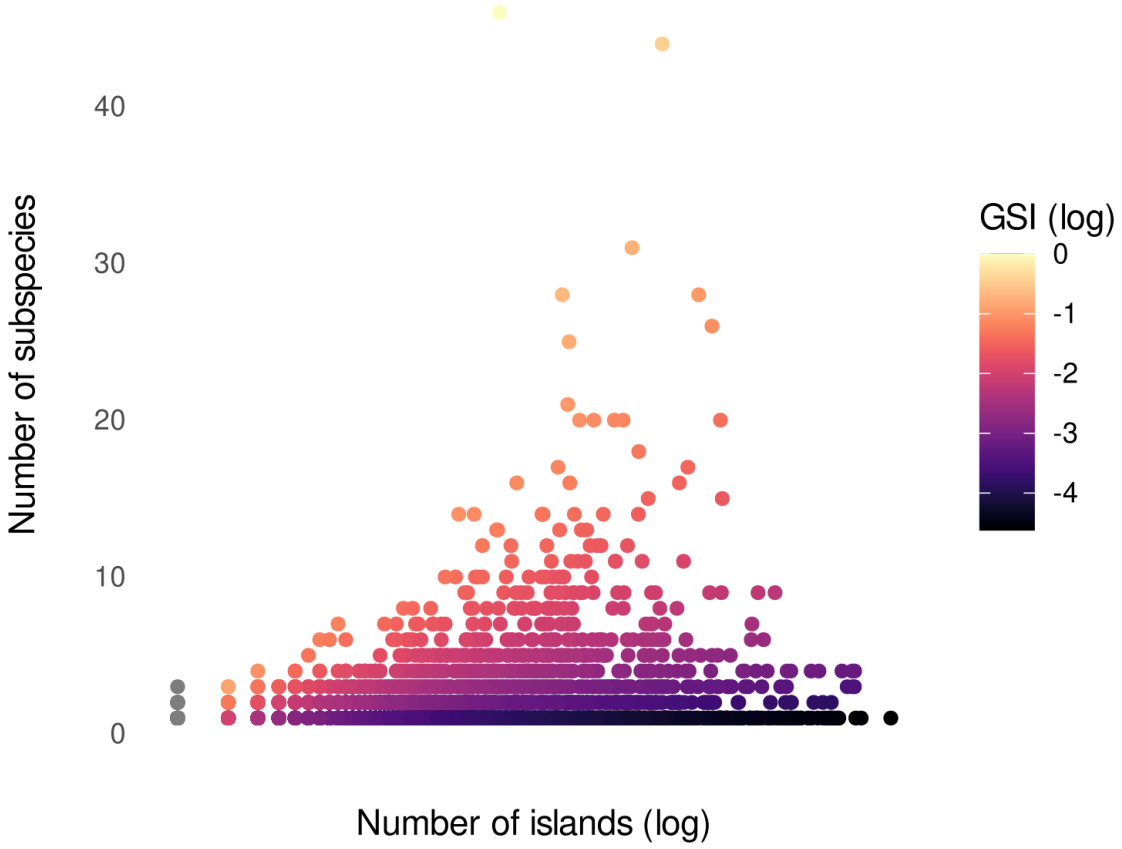


Figure S1. Number of subspecies against the number of islands (log), representing the Great Speciator Index (GSI).

C

Supplemental Material Chapter 3

Supplemental tables can be found at: https:/doi.org/10.5281/zenodo.10252907



Figure S1. Silvereye movement patterns based on banding recovery records between 1956 and 2015. Lines are coloured by distance (normalised) between where the bird was first ringed and where it was caught again. It is worth noting that population density and banding effort vary greatly across space.

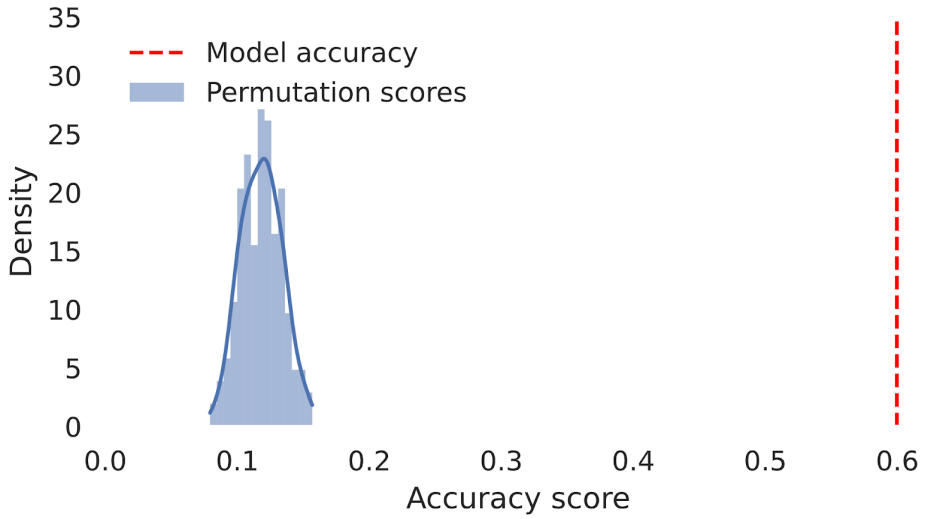


Figure S2. Permutation score test plot representing a distribution of how accurate random classification would be using morphology. The red dashed line represents our model's accuracy in predicting populations based on morphology, indicating an average classification of 60%.

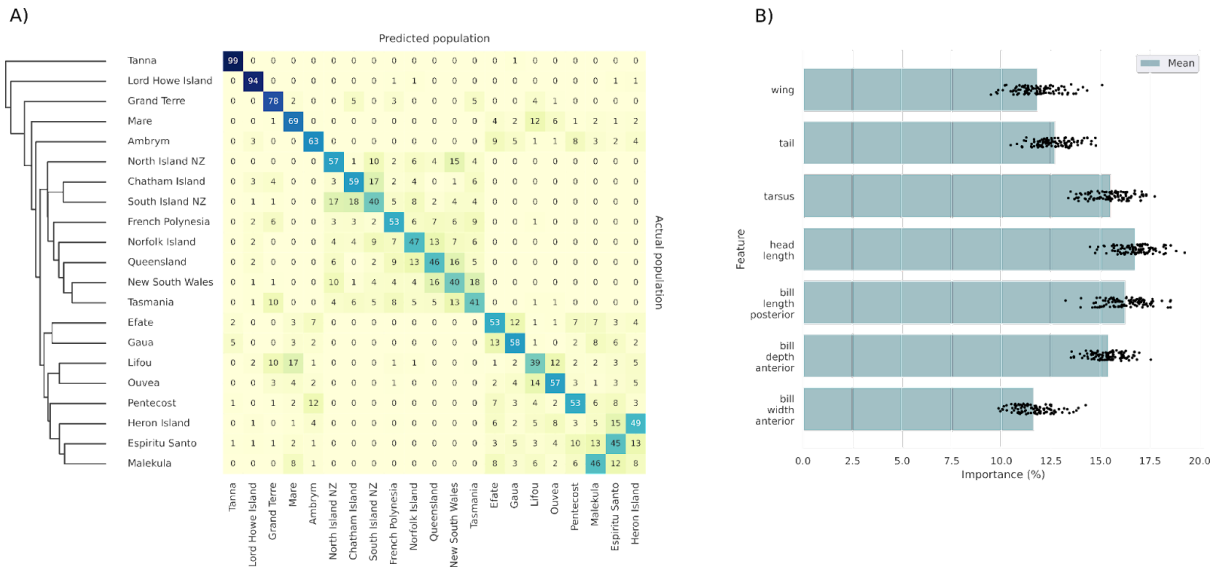


Figure S3. A) Hierarchical clustering of the confusion matrix where each cell is coloured based on the number of times that the actual population was predicted as itself (diagonal) or another population based on morphology. For example, Tanna is highly distinctive so it is predicted as itself 99% of the times while New South Wales only 40% of the times, and it is sometimes incorrectly predicted to be from Tasmania (18%) and Queensland (16%); B) All traits are similarly important in determining the morphology.

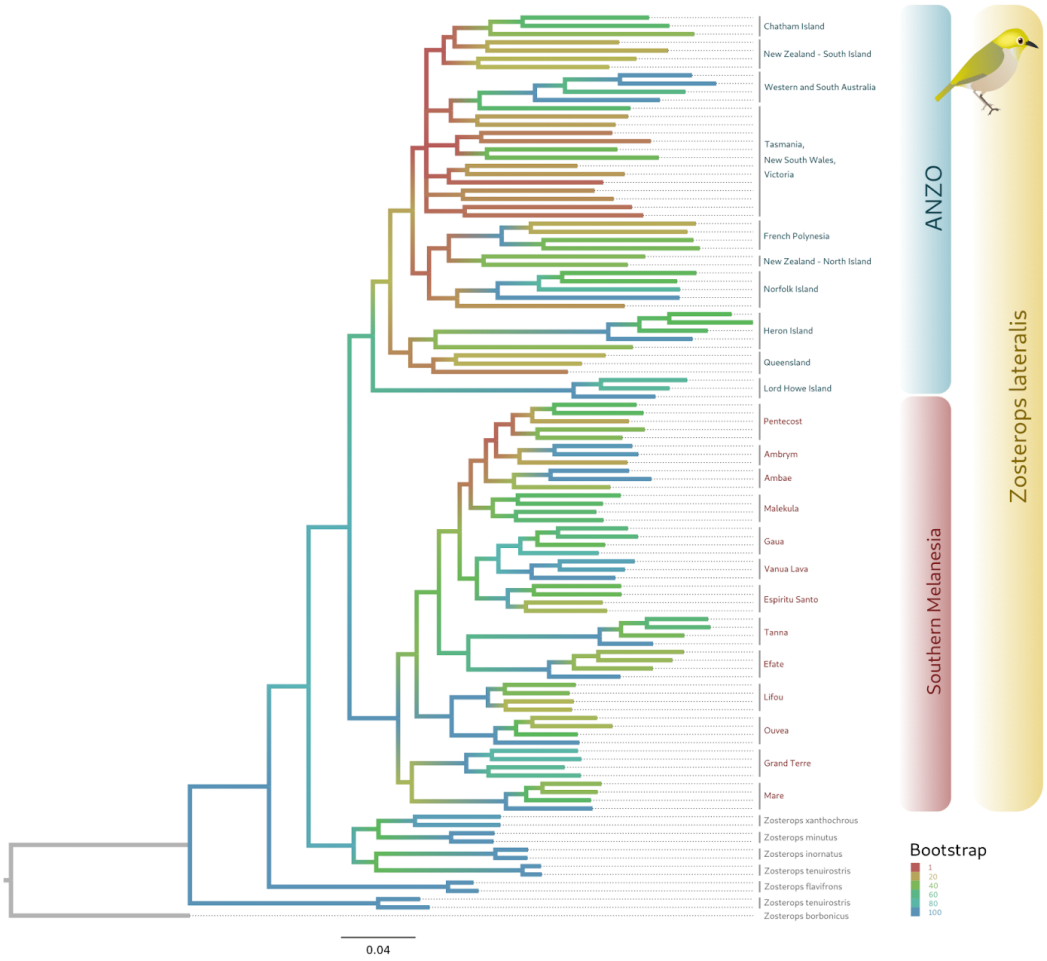


Figure S4. The phylogenetic tree resulting from the IQTREE analysis, where each branch is colour-coded to indicate the support for its parent node. This reveals significant uncertainty across the ANZO cluster (excluding Lord Howe Island) and North and Central Vanuatu.

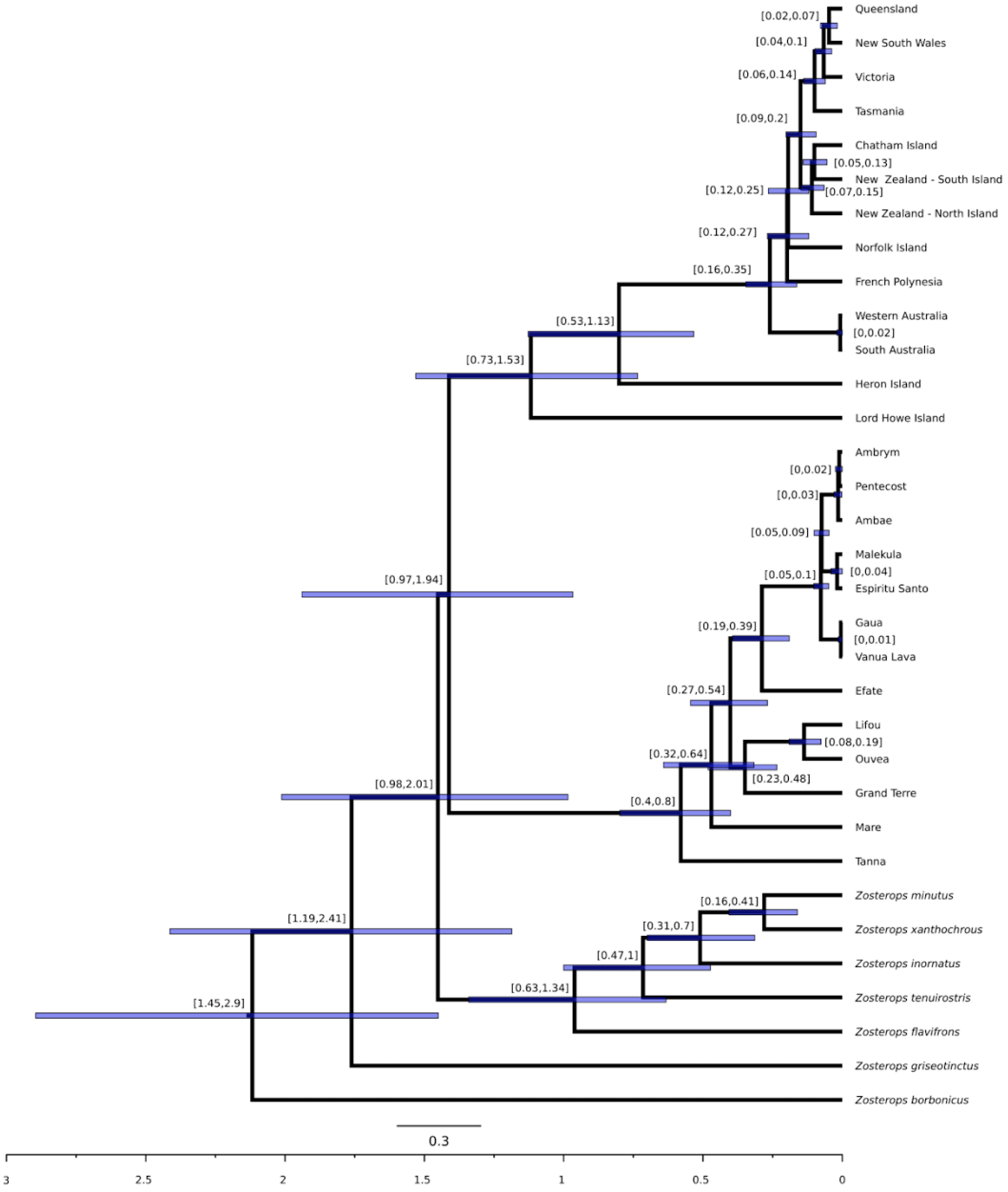


Figure S5. Maximum clade credibility tree representing the consensus topology and the 95% credibility intervals for the age estimate.

D

Supplemental Material Chapter 4

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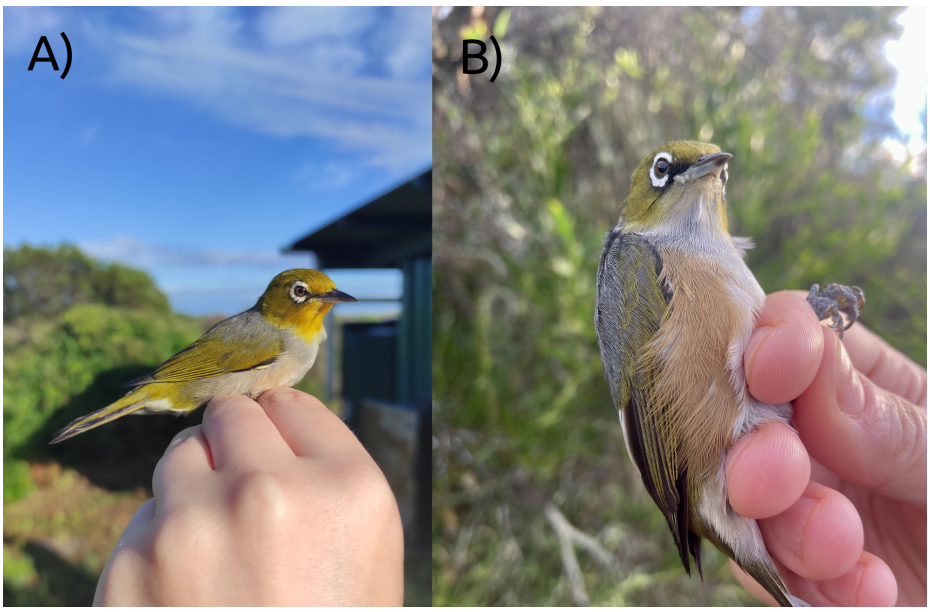


Figure S1. A) *Zosterops lateralis cornwalli* characterised by a bright solid yellow throat and grey flanks. This subspecies is resident on the east coast of the Australian mainland and coastal islands. Picture by Andrea Estandía on Broughton Island, New South Wales B) *Zosterops lateralis lateralis*, characterised by a grey throat and red flanks. This subspecies is found in Tasmania but some individuals migrate to the Australian mainland during winter. Picture by Andrea Estandía in Eagleby Wetlands, Queensland.



Figure S2. NGSadmix results of WGS data for k=2 (left), k=3 (centre), and k=4 (right). k=2 represents the split between the ANZO and SM clusters, k=3 the split within SM: New Caledonia and Vanuatu, although the southern island of Tanna, which is close to New Caledonia, has genetic affiliations with populations from both archipelagos.

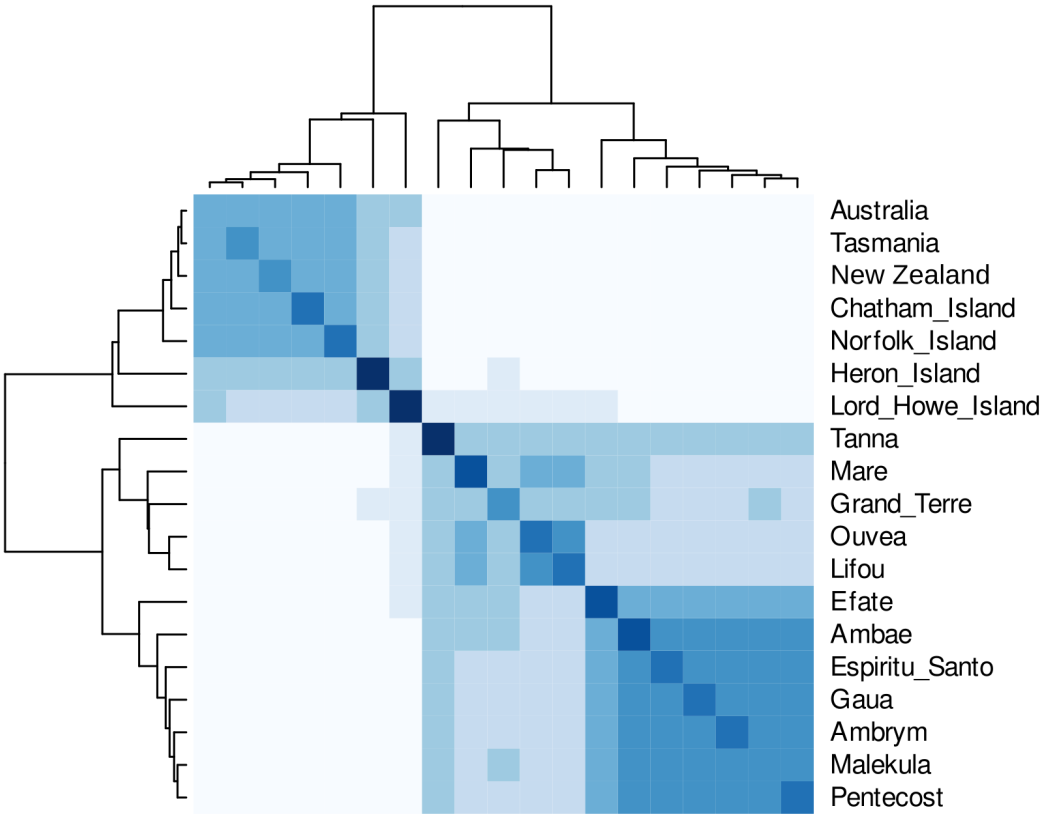


Figure S3. Heatmap produced with the population-level covariance matrix generated with PCAngsd using WGS data. The divergence patterns are consistent with those from NGSadmix.

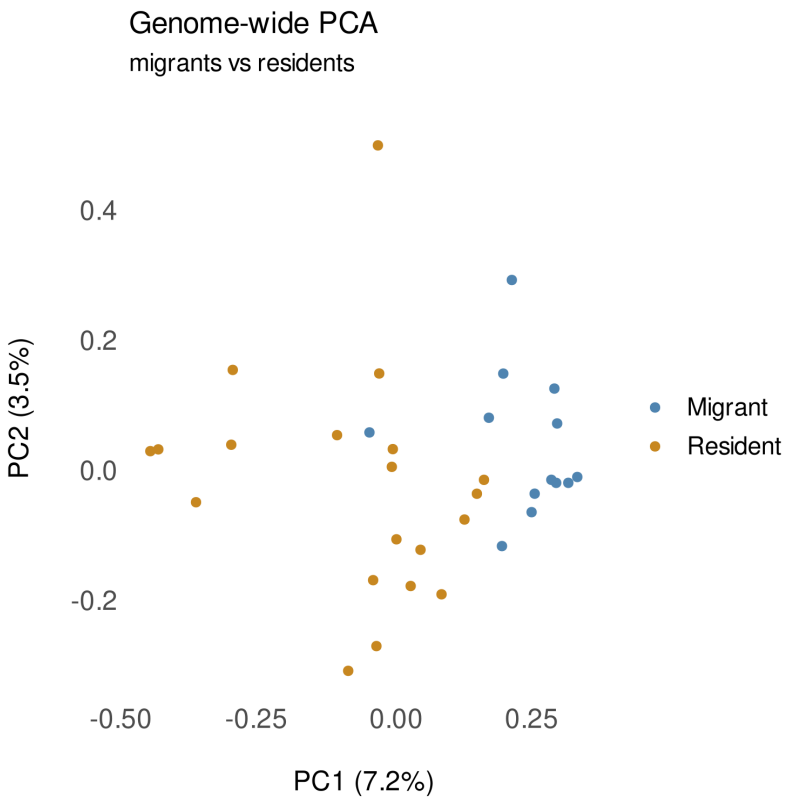


Figure S4. Genome-wide PCA computed with PCAngsd. Individuals are coloured by migratory status as defined in the main text. The variance explained by each component is shown in the axis title.

E

Supplemental Material Chapter 5

Supplemental tables can be found at: https:/doi.org/10.5281/zenodo.10252907

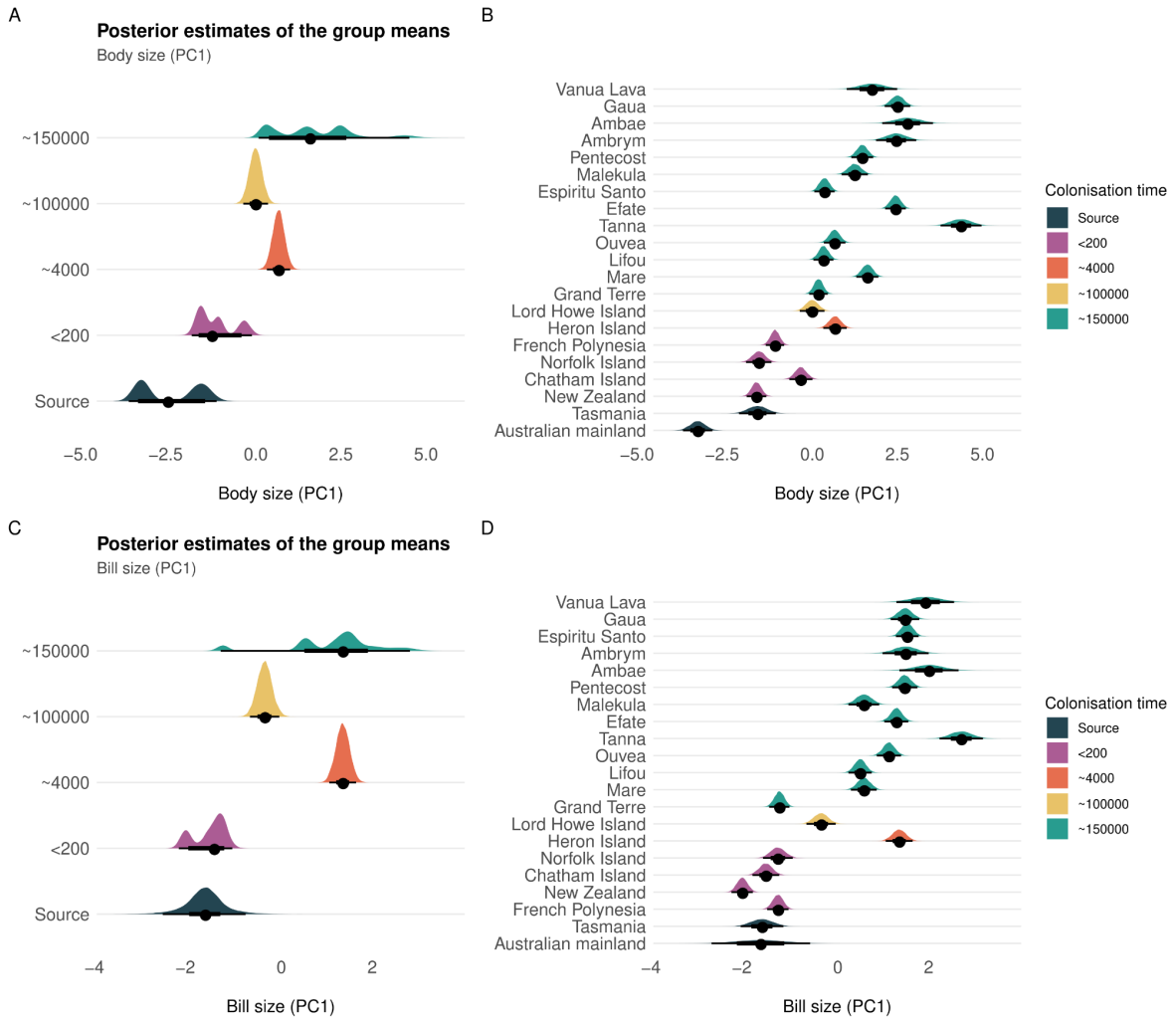


Figure S1. Posterior estimates of the group means obtained from the brms model where increases in body and bill size can be seen for each colonisation.

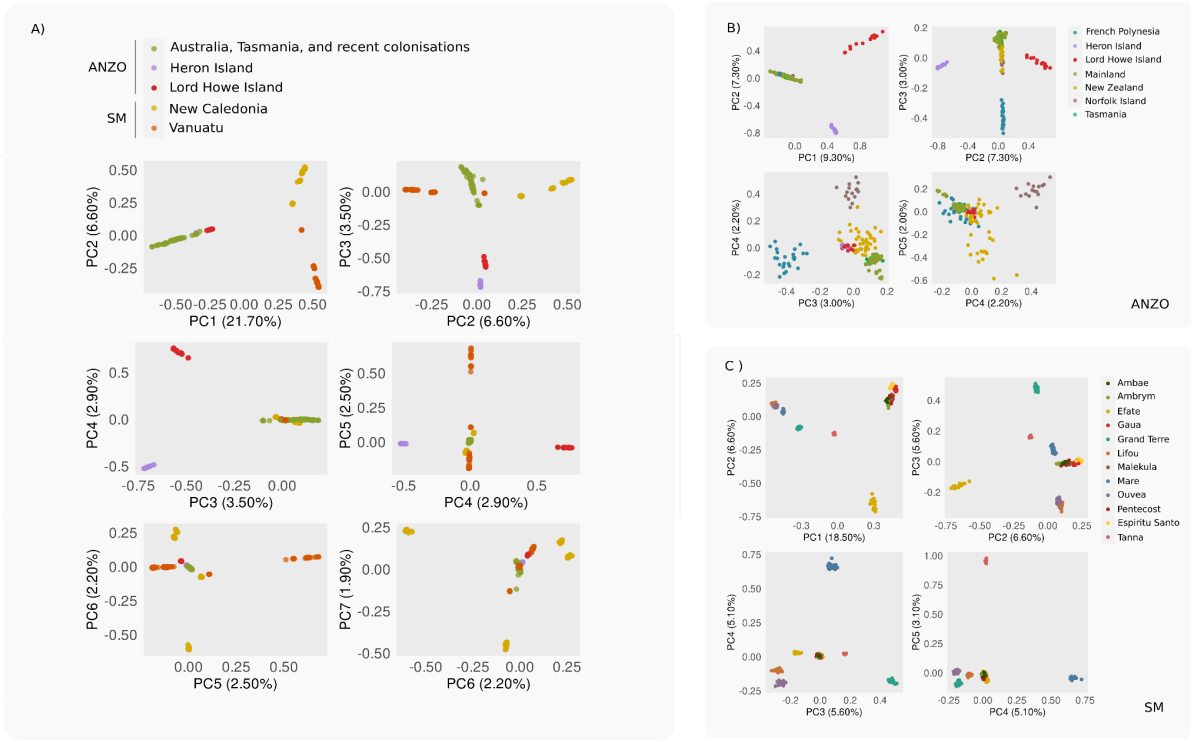
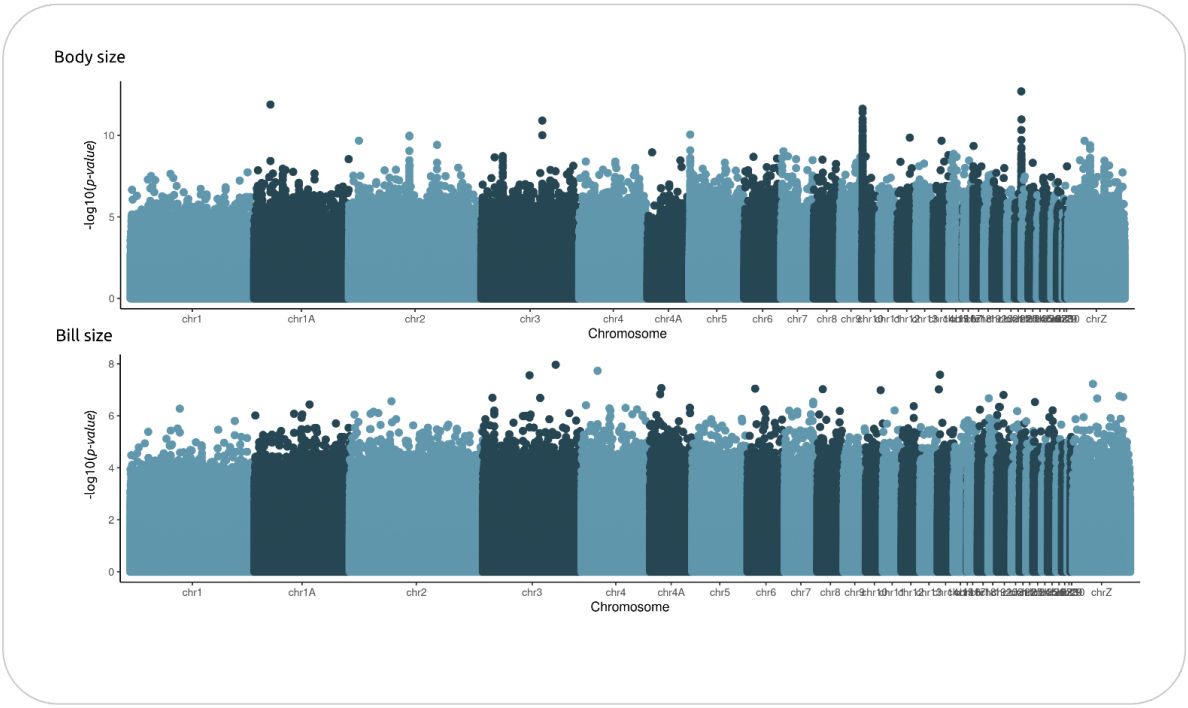


Figure S2. Genomic PCA separating Southern Melanesia (New Caledonia and Vanuatu) and Australia, Lord Howe Island, Heron Island and associated islands in PC1. PC2 separates New Caledonia and Vanuatu.

A) SM



B) ANZO

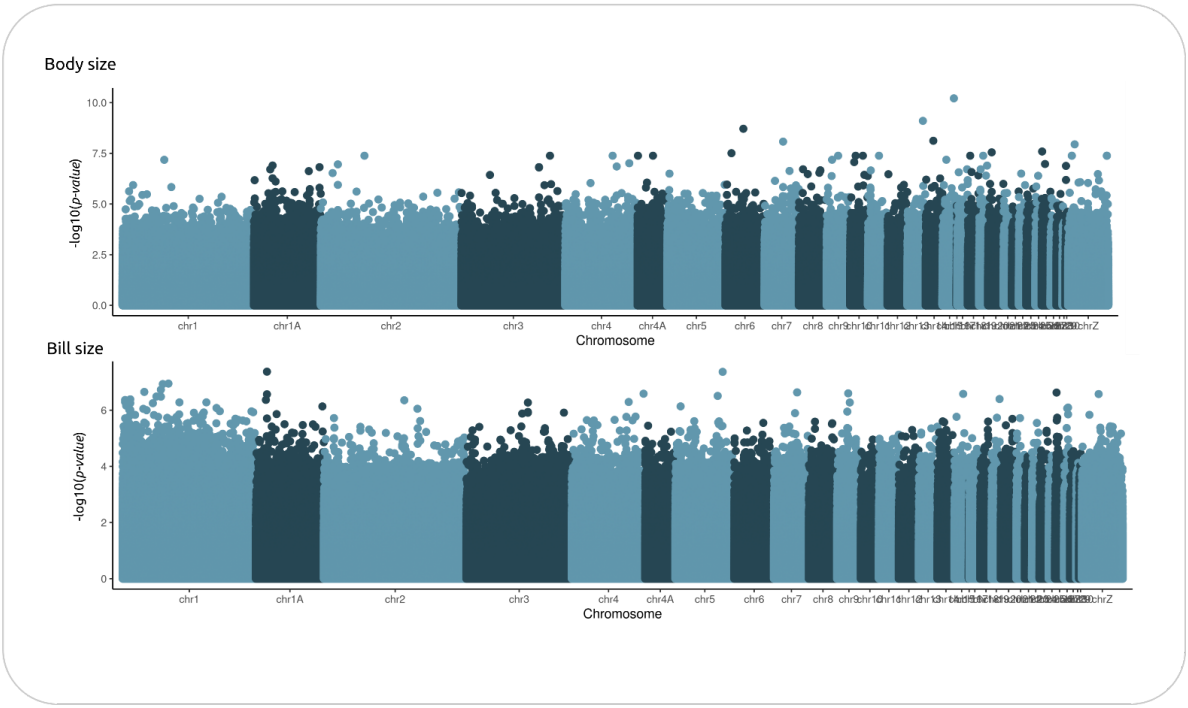


Figure S3. Manhattan plots for body and bill size in A) Southern Melanesia and B) the ANZO cluster

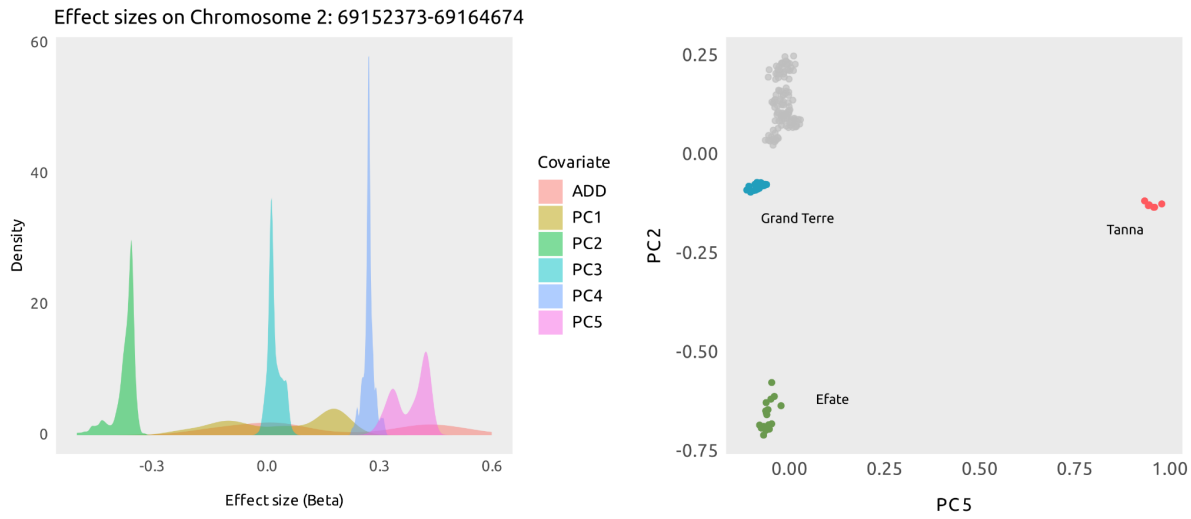


Figure S4. Effect sizes within the association peak for body size on Chromosome 2. Covariates include ADD (effect size of the SNP) and PC1 to PC5 used to control for population structure on the GWAS including only Southern Melanesia. A few SNPs have a greater effect size than any of the population structure eigenvectors, indicating no conflation. PC2 and PC5 also showed high effect sizes, and these represent the separation of Tanna and Efate, the two southernmost islands within Vanuatu.

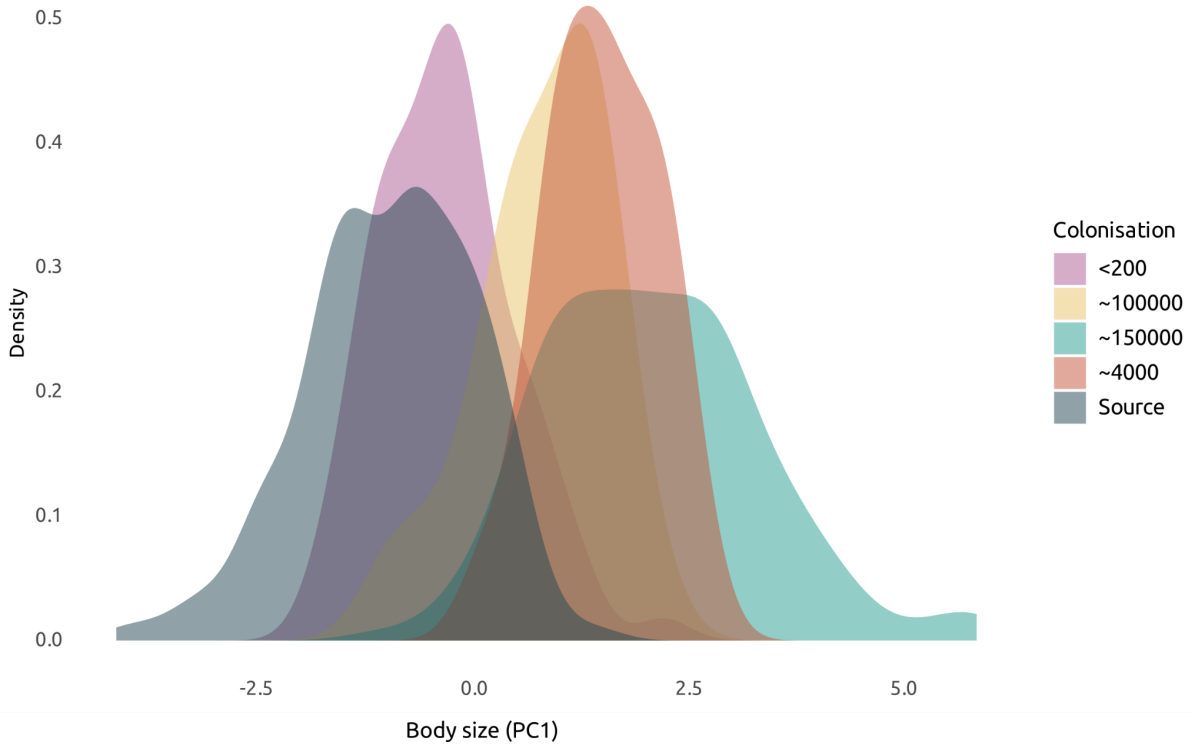


Figure S5. Distribution of body size across colonisations.