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 colonised island populations (< 200 years), compared to evolutionarily older populations. Bayesian broken stick regression models supported a reduction in *CREB1* length with time since colonisation; and iii) like *CREB1*, *DRD4* showed differences in polymorphisms between recent and old colonisations 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: dispersal, diversification, *Zosterops lateralis*, islands, great speciator, *CLOCK*, *CREB1, ADCYAP1, NPAS2, DRD4*

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 (Lester *et al.*, 2007; Sheard *et al.*, 2020; Alzate & Onstein, 2022) 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 (Hedrick, 1983; Slatkin, 1987; Coyne & Orr, 1998; Mayr & Diamond, 2001; Grant & Grant, 2008).

Great speciators are those bird species that inhabit multiple islands reached by overwater dispersal events, suggesting excellent colonisation ability, yet display multiple morphological subspecies (Diamond *et al.*, 1976; Moyle *et al.*, 2009; Pedersen *et al.*, 2018; Manthey *et al.*, 2020). 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 (McNab, 2002; Bonte *et al.*, 2012) 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-colonisation 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 (Duckworth & Badyaev, 2007; Duckworth & Kruuk, 2009; Botero-Delgadillo *et al.*, 2019). 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*) where 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 (Fidler *et al.*, 2007; Steinmeyer *et al.*, 2009; Ruegg *et al.*, 2014; Canestrelli *et al.*, 2016), and migratory behaviour (Johnsen et al., 2007; Peterson et al., 2013; Bazzi et al., 2015; 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

98 (SNPs) and differences in avian personality and migratory propensity (Riyahi et al., 2017;
99 Timm et al., 2019; Sauve et al., 2021; Table S1).

100 The Zosteropidae family (white-eyes, yuhinas and allies) consists of 142 species (Clements *et*
101 *al.*, 2019) many of which are highly dispersive as evidenced by colonisation of numerous
102 oceanic islands throughout the Indian and Pacific oceans, along with the broad continental
103 distributions of some species (Mees, 1969; Clegg *et al.*, 2002). This family shows one of the
104 highest per-lineage diversification rates for vertebrates (Moyle *et al.*, 2009) and divergence
105 can occur even across minor geographic barriers (e.g. water gaps of just 2 km) (Mees, 1969;
106 Glor, 2011; Bertrand *et al.*, 2014; Cowles & Uy, 2019; Manthey *et al.*, 2020). A particularly
107 interesting species within this family is the silvereye (*Zosterops lateralis*), considered a great
108 speciator having multiple subspecies (17 morphological subspecies; Clements et al., 2019)
109 with a wide natural distribution - including the Australian mainland and Tasmania, the North
110 and South Islands of Aotearoa New Zealand (henceforth New Zealand), outlying oceanic
111 islands of Australia and New Zealand, and the archipelagos of New Caledonia, Vanuatu, and
112 Fiji (Fig. 1A). Silvereyes also display a variety of gene flow potentials: the Tasmanian
113 subspecies (*Z. l. lateralis*) is a partial migrant (Mees, 1969); those in the central Vanuatu
114 archipelago display high levels of outgoing gene flow, while more peripheral populations
115 have high levels of incoming gene flow (Clegg & Phillimore, 2010); and others, such as on
116 Heron Island and Lord Howe Island in the southern Great Barrier Reef, are sedentary and
117 genetically isolated (Sendell-Price *et al.*, 2020).

118 Here, we assess variation in six personality-related candidate genes in the silvereye at three
119 different levels: individuals, populations and subspecies. Our aim is to determine whether
120 there are signatures consistent with a genetic switch that would explain rapid shifts in
121 dispersal, leading to reduced gene flow and subsequent increased opportunity for divergence.
122 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 colonisation; and iii) if candidate gene variation is correlated with individual migratory status in the partially migratory Tasmanian population.

Methods

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 (Fig. 1B), and Southern Melanesia (New Caledonia and Vanuatu) referred to henceforth as SM (Fig. 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 (Fig. 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 (10mM EDTA pH 8.0, 10mM TRIS-HCl pH 8.0, 20mM NaCl, 1% SDS, Seutin et al., 1991).

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 10ng 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).

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 10ng DNA template, 1.0 unit BioTaq (Bioline USA Inc.), 1 x BioTaq reaction buffer, and 0.5µM each forward and reverse primer, 200µM dNTPs, 1.5mM $MgCl_2$, 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 sec, and an extension step of 72˚C for 2 min. This was followed by 25 cycles of 94˚C for 30 sec, 55 ˚C annealing step for 30 sec, 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.

Gene flow estimates

As a proxy of population dispersiveness, we estimated outgoing gene flow rates from each population. We used whole genome sequences 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 generated 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 visualised 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 estimations were not a biased product of the SNP selection, we created five independent datasets of randomly selected 10,000 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±1.96*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.

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 colonised 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:

(a) 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

245 located island population with moderate outgoing gene flow to many close islands. *DI* was
246 scaled from 0 (non-dispersive) to 1 (maximally dispersive).

247 $$DI = \sum g_{\mathrm{i}\ ->\ \mathrm{j}} * d_{\mathrm{i}\ <->\ \mathrm{j}}$$

248 Equation 1

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

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

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

263 a) Differences in length among populations for each candidate microsatellites

264 b) Bayesian linear mixed models:

265 ◦ migration: *CLOCK* only

266 ◦ 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 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 10bp in other birds (Bourret & Garant, 2015; Bazzi *et al.*, 2017). 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).

Results

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) (Fig. 2; Fig. 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 (Fig. 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 (Fig. 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, Fig. 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 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 (Fig. S4), suggesting a lack of general genome-wide divergence.

Variation in candidate microsatellites, DRD4 and SERT

Mean and individual allele lengths for microsatellite candidate loci are shown in Figure 2 and Figure 3. *CREB1* showed longer mean lengths for the Australian mainland, Tasmania (migrants and non-migrants), and the recently colonised populations of New Zealand,

Chatham Island, and Norfolk Island (Fig. 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 (Fig. 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) (Fig. 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 (Fig. 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 (Fig. 3C; Table S7.4).

We identified ten *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.

Candidate gene association tests

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

(Fig. S4; Tables S9.1-S9.2). Time since colonisation 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; Fig. 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; Fig. 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.

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-colonising 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 colonisation and dispersal propensity, with recently colonised 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 colonised 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 colonised populations and shorter in island populations colonised 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 (551bp) 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 colonisations converge in a reduction in length, suggesting that it is unlikely that just by neutral processes each of these independent colonisations 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 colonisations (sedentary genotype). This is because the colonising 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

387 acted onto this phenotypic variation and associated genotypes. Another mechanism by which
388 the same phenotype could have arisen is through new mutations in each of the colonisations.
389 However, this scenario is unlikely because standing variation is a more relevant mechanism
390 driving parallel evolution at shallow timescales (Hedrick et al. 2013).
391 At the broad scale, the most dispersive populations, and those evolutionarily recent
392 colonisation events, carry longer allele lengths, compared to more sedentary, and more
393 ancient island populations. This is the opposite pattern to that found in common buzzard
394 (*Buteo buteo*) where non-dispersive individuals carried longer *CREB1* allele lengths
395 (Chakarov *et al.*, 2013). *CREB1* allele lengths have also been shown to correlate with other
396 life-history traits that also depend on circadian and circannual clocks e.g. incubation duration
397 (Bourret & Garant, 2015) and male moult speed (Bazzi *et al.*, 2017). The broad scale
398 patterns, and suggested association with dispersal, were not always reflected within each of
399 the longer- and shorter-allele length groups. For instance, within SM, Gaua and Espiritu
400 Santo displayed the longest lengths, but according to our gene flow analyses, Espiritu Santo
401 was a dispersive population within its grouping but Gaua was not; likewise for Norfolk Island
402 in the ANZO cluster.

403 While the obvious length variation pattern seen at *CREB1* appears to have biological
404 relevance for dispersal propensity in our study system, a mechanistic resolution of the
405 paradox of the great speciators also requires dispersal shifts to be rapid (Diamond *et al.*,
406 1976). We were unable to pinpoint when the *CREB1* allele size reduction occurs because of a
407 lack of island populations of intermediate ages. At best we can say that for silvereyes, a shift
408 in dispersal propensity is relatively rapid, taking more than 63 to 95 generations (South Island
409 New Zealand, 190 years since colonisation, generation time of 2 to 3 years) and less than one
410 to two thousand generations (Heron Island, 3,000 to 4,000 years old) (Clegg et al., 2008).

411 Variation at a second gene, *CLOCK*, was associated with migratory status of individuals in a
412 partial migrant population, with migrant individuals having longer *CLOCK* allele variants,
413 including long variants absent in non-migrant individuals from Tasmania, Australian
414 mainland and all island populations. Migrant and sedentary individuals did not show genome-
415 wide population structure, suggesting that *CLOCK* could potentially be a gene under
416 divergence. Previous studies in migratory birds focusing on *CLOCK* variation show mixed
417 results. Longer allele lengths have been found to be positively associated with migratory
418 propensity (Peterson *et al.*, 2013), and variation in the phenology of migratory species
419 (Liedvogel *et al.*, 2009; Caprioli *et al.*, 2012; Bazzi *et al.*, 2015), but in some cases allele
420 lengths are negatively correlated with migration date (Ralston *et al.*, 2019) or not correlated
421 at all (Mueller *et al.*, 2011; Contina *et al.*, 2018; Parody-Merino *et al.*, 2019).
422 *CLOCK* plays a key role in regulating the circadian oscillator gene complex (Panda *et al.*,
423 2002), and is associated with variation in the phenology of photoperiodic traits (e.g.
424 migratory behaviour) (Table S1). For example, in migratory equatorial African stonechats
425 annual-cycle timing is associated with *CLOCK* length (Justen *et al.*, 2022). Increasing
426 photoperiod can initiate migration (Robart *et al.*, 2018; Assadi & Fraser, 2021). In silvereyes,
427 photoperiodic stimulation experiments resulted in migratory restlessness being triggered only
428 in migratory caged birds but not in resident ones, supporting a genetic link with migratory
429 behaviour (Chan, 1994). Consequently, photoperiod changes could be the migration trigger
430 with the onset of shorter autumnal days. The Tasmanian population is one of the few
431 silvereye migrant populations situated where changes in day length during the non-breeding,
432 winter period is substantially greater than changes experienced further north and closer to the
433 equator. Another location where these conditions are met is New Zealand. In fact, even
434 though records are few, large flocks of silvereyes seem to have migrated from the South to
435 the North Island (Dennison *et al.*, 1987), providing further support that photoperiod changes

436 which are mediated at least partially by genetics, can lead to migration. Some overwater
437 dispersal events might be a direct consequence of off-course migration. For example, the
438 recent sequential colonisation sequence of the silvereye from Tasmania to New Zealand and
439 outlying islands was likely initiated by an off-course flock of Tasmanian migrants (Mees
440 1969). However, Tasmanian migrants sampled in this study carry long *CLOCK* allele variants
441 that were not recovered in the recently colonised populations. This could because we are not
442 sampling the full allelic diversity of *CLOCK* in recently colonised populations (New Zealand
443 (n=6), Chatham Island (n=13), Norfolk Island (n=2); Table S3), and/or underlying effects of
444 a different set of genes that we have not included in this study on dispersal ability in these
445 populations. The genetic basis of partial migration in Tasmanian silvereyes and whether
446 associated variants are present in recently colonised populations should be further explored in
447 future work using a hypothesis-free genome-wide approach.
448 *DRD4* is one of the most well-studied candidate genes related to exploratory and risk-taking
449 behaviour (Bubac et al. 2020) – both of which could feasibly have links to dispersal
450 propensity. We found a single polymorphism within *DRD4* (SNP83) that showed fixed
451 differences between two young (more dispersive) and seven old (more sedentary) island
452 populations. The Tasmanian population was polymorphic (G and A represented), the recently
453 established populations of Chatham Island and South Island New Zealand were fixed for A,
454 and Southern Melanesian populations fixed for G. SNP83 is located in intron 2 and
455 corresponds to base pair 9,423 on the *DRD4* orthologue of the great tit (Fidler et al., 2007). It
456 has not been noted previously as having any phenotypic associations. Previous work
457 assessing *DRD4* variation and personality in the great tit focused on associations between
458 exploratory behaviour and variation at ‘SNP830’, revealing large effects in certain
459 populations but not in others (Fidler *et al.*, 2007; Korsten *et al.*, 2013; Riyahi *et al.*, 2017),
460 however SNP830 was not variable in our dataset. Our data adds 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 Liedvogel *et al*., 2009; Liedvogel & Sheldon, 2010; Bourret & Garant, 2015) 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.

Maintenance of high dispersal propensity in a continental island population

Despite being an old insular form (>200K 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 colonisation 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).

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 characterised 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 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.

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 colonisation. 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.

Figure captions

Figure 1. A) Distribution range of the silvereye highlighted in blue. Red dots represent single islands or archipelagos where multiple islands have been colonised from the Australian mainland or Tasmania. Approximate colonisation times are shown. French Polynesia is shown as inset; Locations of silvereye populations sampled for candidate gene variation: B) ANZO cluster C) SM populations.

Figure 2. A) NGSadmix plot (k = 2) showing a split between ANZO and the SM populations. B) The average *CREB1* allele length per population indicates a reduction in length for Heron

533 and Lord Howe Islands, and all populations in the SM cluster. *CREB1* was not screened for
534 Ambae and Pentecost samples.

535 Figure 3. Individual mean allele lengths (shadowed dots) with mean and standard error for
536 each population (large dots) for the four microsatellite candidate genes. Blue and grey dots
537 correspond to two population genetic clusters identified from admixture analysis. A) *CREB1*
538 is longer in the source populations (Australia and Tasmania), and recently colonised
539 populations (New Zealand, Chatham Islands and Norfolk Island) compared to older
540 populations (Heron Island, Lord Howe Island, and SM populations). B) Migrant individuals
541 highlighted in orange have longer allele lengths at *CLOCK. C) ADCYAP1* shows extensive
542 variation, including shorter lengths in Lord Howe Island and Ambrym and longer lengths in
543 New Zealand, Ouvea and Gaua. D) *NPAS2* shows little variation with southern Vanuatu
544 populations (Erromango, Efate and Tanna) showing an increased mean allele length.

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

549 Figure 5. Broken-stick regression model (*mcp*) for *CREB1* allele length variation. ANZO
550 population data points (triangles) and SM population data points (circles); 100 posterior
551 draws (grey lines); change point posterior distributions (four blue density curves, each
552 representing one chain); and 89% prediction intervals (grey dashed lines). A) Relationship
553 with population age, showing a higher mean length in recently colonised populations and
554 their Tasmanian source population. Change point posteriors range from ~200 years ago to
555 ~4000 years ago. B) Relationship with dispersal index (DI). The highest DI corresponds to
556 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.

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