Using conservation genetics to prioritise management options for an endangered songbird.

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Abstract

Genetic data can be highly informative for answering questions relevant to practical conservation efforts, but remain one of the most neglected aspects of species recovery plans. Framing genetic questions with reference to practical and tractable conservation objectives can help bypass this limitation of the application of genetics in conservation. Using single nucleotide polymorphism genotype data, we conducted a genetic assessment of remnant populations of the endangered forty-spotted pardalote (*Pardalotus quadragintus*), a songbird endemic to Tasmania, Australia. Our objectives were to inform strategies for conservation of genetic diversity in the species, estimate effective population sizes and patterns of inter-population movement to identify management units relevant to population conservation and habitat restoration. We show previously undetected population genetic structure and identify two small populations on mainland Tasmania as 'satellites' of larger Bruny Island populations connected by migration. Our data identify management units for conservation objectives relating to genetic diversity and habitat restoration. A prioritisation analysis showed that two populations, North Bruny Island and Maria Island, are most important for representing the genetic diversity in forty-spotted pardalotes. Although our results do not indicate the immediate need to genetically manage populations, the small effective population sizes we estimated for some populations indicate that they are vulnerable to genetic drift, highlighting the urgent need to implement habitat restoration to increase population size. We discuss how our genetic assessment can be used to inform translocation trials and show that by assessing contemporary genetic aspects, valuable information for conservation planning and decision-making can be produced to guide actions that account for genetic diversity and increase chances of species recovery.

Introduction

Genetic diversity is critical for maintaining evolutionary potential and resilience, allowing species to adapt to environmental changes (Willi *et al.* 2006; Markert *et al.* 2010; Smith *et al.*

2014). As small and fragmented populations are prone to genetic processes that can increase extinction risk (e.g. inbreeding depression; Carlson *et al.* 2014; Smith *et al.* 2014), incorporating genetic factors in the management of endangered species can inform actions to avoid loss of genetic variation, increasing the chances of recovery (e.g. Ottewell *et al.* 2016; Weeks *et al.* 2017). However, despite its importance, genetic parameters and genetic management remain one of the most neglected aspects in species recovery plans (Laikre *et al.* 2010; Taylor *et al.* 2017; Ralls *et al.* 2018). This gap stems in part from challenges in translating genetic information into management actions (Taylor, Dussex, & van Heezik, 2017). Furthermore, conservation practitioners and conservation genetics researchers may sometimes lack a clear understanding of how genetic tools can assist in practical conservation efforts, resulting in missed opportunities for better informed management decisions.

Genetic data can answer questions relevant to conservation efforts (Manel *et al.* 2003; Frankham 2010). For example, dispersal and connectivity are difficult to measure directly by studying marked individuals at large spatial scales, but genetic techniques can overcome this challenge (Koenig *et al.* 1996; Manel *et al.* 2005; Schwartz *et al.* 2007). Understanding population connectivity is crucial to define "management units" - population units identified within species to help guide management and conservation (Fraser & Bernatchez, 2001; Palsbøll *et al.* 2007; Funk *et al.*, 2012). The two most common units used in conservation are evolutionarily significant units, representing populations that need to be managed separately because of high genetic and ecological distinctiveness (Allendorf *et al.* 2012), and management units, which are defined as demographically independent populations (Moritz, 1994; Funk *et al.*, 2012). However, we argue that there cannot be a single genetic definition of a management unit because their definition should account for the management question. Here we define management units based on both genetic diversity and dispersal patterns. These management units can inform the appropriate scale for demographic and genetic monitoring, or identify the likely origins of recruits for population growth following conservation initiatives. In addition to understanding population

dynamics, quantifying genetic diversity within and among populations for management is crucial because these parameters play a key role in fitness and population viability (Frankham, 2010).

Assessing genetic parameters can help prioritize populations for conserving genetic diversity and inform translocation strategies. For example, populations with high genetic diversity might be particularly important for conservation and those with low diversity might require genetic management (e.g. augmenting gene flow; Frankham *et al.* 2019). In species for which conservation translocation (i.e. movement of organisms from one site to another; IUCN/SSC 2013) is advised, a genetic assessment can inform where founders should be sourced from to guarantee genetic representation and for planning reinforcement regimes to increase effective population size and preserve genetic diversity (Weeks *et al.*, 2015). By conducting a genetic assessment, conservation practitioners can implement genetic management if required, or focus on strategies that mitigate threats and factors limiting population growth with the aim of increasing effective population size (e.g. habitat restoration; Ottewell *et al.* 2016).

Here, we use genomic data to investigate the population biology and genetic diversity of an endangered songbird to inform conservation management. The forty-spotted pardalote *Pardalotus quadragintus* is a habitat specialist endemic to the island state of Tasmania, Australia. The species relies on tree cavities for nesting and forages primarily on one tree species, the white gum (*Eucalyptus viminalis*). Forty-spotted pardalotes have been extirpated across most of their former range, mainly due to deforestation and habitat degradation (Brown, 1986; Threatened Species Section, 2006), and are now largely confined to two offshore islands (Bruny and Maria Islands) and two small patches of forest on coastal mainland Tasmania close to the Bruny Island population (Fig. S1). We collected DNA samples across known remaining populations and used genotyping-by-sequencing to undertake a comprehensive genetic assessment of the species. Because of the fragmented nature of remaining populations, we expected them to show genetic structure, and based on this prediction, we aimed to address the following questions: How is genetic diversity distributed within and among populations? Do some populations have lower

genetic diversity than others? Which populations are most important for maintaining genetic diversity in the species? How many management units are necessary to conserve genetic diversity and facilitate recovery? What is the effective population size (N_e) within management units? Are the smaller populations adjacent to Bruny Island discrete populations, and possibly at risk of extinction, or are they connected by migration? Answering the latter question will clarify the extent to which the species can colonise new areas if habitat and threatening processes are better managed. By answering these questions, we can make recommendations on management strategies that account for genetic diversity and the harmful genetic effects common in small populations.

Methods

Study species and sample collection

The forty-spotted pardalote is a small, cavity nesting passerine endemic to Tasmania, Australia. The species' occurrence is positively correlated with the abundance and age of their primary food tree (white gum; Alves *et al.* 2019). Threats limiting remaining populations include ongoing habitat degradation, low nest site availability, competitors, and parasitism by the larvae of an ectoparasitic fly (*Passeromyia longicornis*) that causes severe nestling mortality (Threatened Species Section 2006; Edworthy 2016; Edworthy *et al.* 2019). We collected samples from three offshore Islands (Bruny Is., Maria Is. and Partridge Is., a small island just offshore from the southwestern coast of Bruny Is.) and from two sites on mainland Tasmania (Tinderbox Peninsula and Southport, where a small population was rediscovered in 2015; Fig. 1). South Bruny Is. and North Bruny Is. are isolated by a long and narrow isthmus (~7 km) of inhospitable habitat, so we treated them as separate population units. Maria Is. is also divided by an isthmus (700 m), but it is smaller and supports pardalote habitat, so we did not consider populations on either side of the isthmus to be geographically isolated. These sites comprise all known remaining populations of

the species (Fig. 1), with the possible exception of Flinders Island in the Bass Strait where pardalotes were last recorded in 2012 at very low numbers (Webb *et al.*, 2019) and may be functionally extinct.

We sampled nestlings and adult birds between 2012 and 2019. Nestling samples were collected during nest monitoring for other studies (i.e. Edworthy *et al.* 2019; Alves *et al.* 2020) and adult birds were captured using mist-nets. To ensure the same individual was not resampled, we banded all birds using bands supplied by the Australian Bird and Bat Banding Scheme. We collected blood using brachial venepuncture in accordance with guidelines approved by an Australian National University Animal Ethics Permits 2012/34 and A2017/38 and Tasmanian Government Scientific Permits TFA13956, TFA14295, and TFA18255. Blood was stored either in 70% ethanol or on FTA paper (WhatmanTM). We collected 316 samples across the populations (Maria Is. n = 79, North Bruny Is. n = 172, South Bruny Is. n = 25, Partridge Is. n = 12; Southport n = 3, Tinderbox Peninsula n= 25).

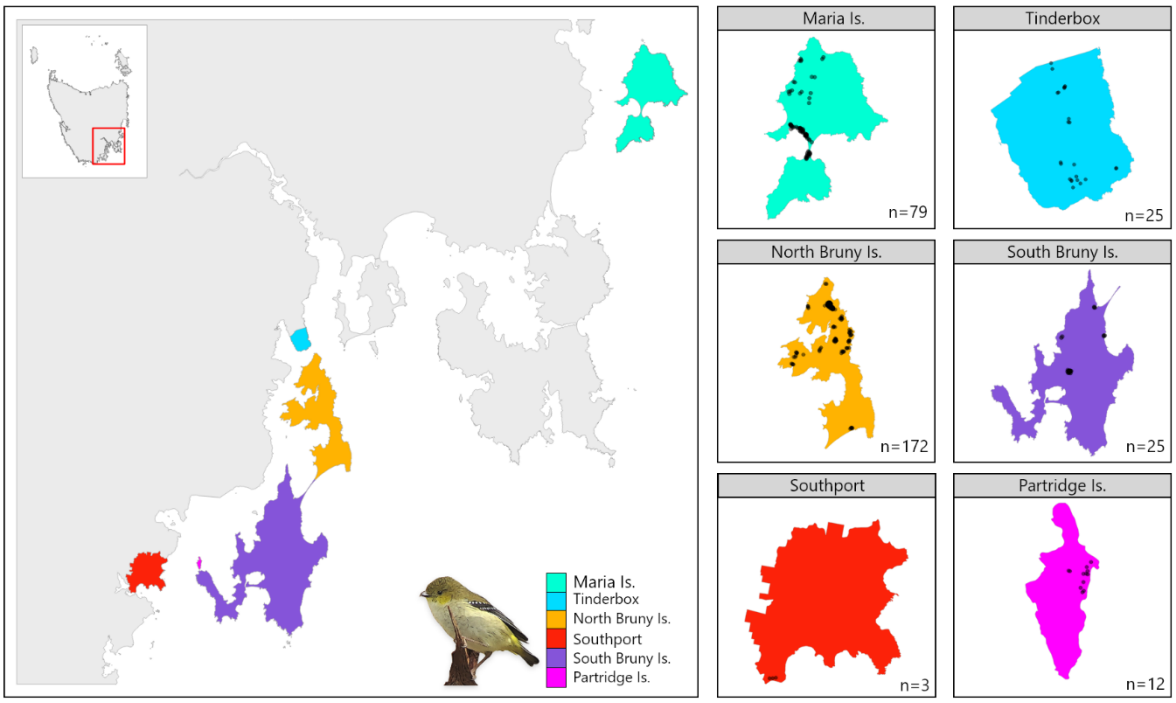

Figure 1. Spatial distribution of the six sampled forty-spotted pardalote populations in eastern Tasmania. Small panels on the right show each location with sample size and black dots represent sample locations.

DNA extraction, SNP genotyping and exploratory analysis

DNA extraction and SNP (single-nucleotide polymorphism) genotyping were performed by Diversity Arrays Technology Pty. Ltd. (DArT; Canberra, Australia; https://www.diversityarrays.com/), using DartSeq™ protocols (Jaccoud *et al.* 2001; Kilian *et al.* 2012). DartSeq is a genome complexity reduction technology that digests genomic DNA using pairs of restriction enzymes (cutters). We first plotted histograms of basic SNP metrics including call rate (i.e. the proportion of non-missing data per SNP), repeatability based on technical replication of 30% of samples and sequencing depth, and used scatterplots to visually assess whether these locus-level metrics were associated with expected and observed heterozygosity and F_{IS} within the larger populations sampled (Figs. S2, S3, S4). We then filtered the data using the 'dartR' package in R (Gruber *et al.*, 2018; R Core Team 2021). We filtered SNPs by repeatability (100% repeatability), removing monomorphic loci, minimising missing data (per-locus call rate > 0.95), dropping secondary SNPs in same sequence (retaining the SNP with highest polymorphic information content) and on pairwise Hamming distance (i.e. loci with trimmed sequence tags that are too similar). We also filtered individuals by amount of missing data (call rate > 0.90). DArT's sequence processing and SNP-calling produced 33,831 single-nucleotide polymorphism (SNP) loci, of which 4,368 SNPs were retained after filtering (no individual samples were dropped during filtering). Using the filtered dataset, we calculated overall genetic diversity statistics (i.e. H_o, H_e, F_{ST}, F_{IS}) and pairwise F_{ST} with 100 bootstraps. The filtered dataset was used in all subsequent analyses.

Population genetic structure

As an exploratory step, we conducted a principal coordinate analysis (PCoA) using package 'dartR' (Gruber *et al.*, 2018) to visualise underlying population structure. Then, we further investigated population structure using two methods. We first conducted a discriminant analysis of principal components (DAPC) using package 'adegenet' v.2.0.0 in R v. 4.0.2 (Jombart 2008; Jombart & Ahmed 2011; R Core Team 2020). DAPC identifies and describes clusters of genetically related individuals from large datasets by summarizing the genetic differentiation between groups, while overlooking within-group variation, therefore achieving the best discrimination of individuals into pre-defined groups (Jombart, Devillard, & Balloux, 2010). We first assumed 1 - 6 clusters (*K*) and used Bayesian Information Criterion (BIC) to assess the best supported model and identify the optimal value of *K* to evaluate population groupings (Jombart, Devillard, & Balloux, 2010).

We also analysed population structure using the Bayesian clustering method in the STRUCTURE program (Pritchard, Stephens, & Donnelly, 2000) implemented via the R package strataG (Archer, Adams, & Schneiders, 2017). Using the *structureRun* function, we ran two models, one in which we assumed correlated allele frequency among populations (which is realistic if there is some gene flow), and one assuming uncorrelated allele frequency, which may have higher power to detect low levels of structure (Pritchard, Wen, & Falush, 2009). For both models, the remaining parameters were the same. We used a burn-in period of 20,000 and 80,000 MCMC iterations, and used the admixture model. STRUCTURE can yield erroneous inferences when samples from different populations are uneven (e.g. Kalinowski 2011; Puechmaille 2016), but changing some parameters can overcome this issue (Wang, 2017). Since our sample was unbalanced, we followed Wang (2017) and set the parameter alpha to 1.0/K (1.0/6 = 0.16; six "populations" in our case), and used the alternative ancestry prior (unipriorialpha = 0). We ran analyses for K = 2 to 6, each replicated five times and used diagnostic plots of number of groups (K) and first and second order changes in LnP(K) as described in Evanno *et al.* (2005) to choose the most likely number of clusters. We then used function CLUMPP in

strataG (Archer, Adams, & Schneiders, 2017) to average the results and minimize variance across iterations (Jakobsson & Rosenberg, 2007).

Identification of immigrants

To identify individual origin and investigate possible source-sink dynamics we conducted an individual genetic assignment analysis using the R package 'rubias' (Moran & Anderson, 2018). We used the function "self-assign" which assigns individuals to the population of origin using a *leave-one-out* procedure. Working through each individual in the dataset, the method removes an individual and uses the remaining individuals as a reference panel to calculate the likelihood of that genotype arising in every possible source population. It reports likelihoods for 'reporting units', which can be the same as the candidate source populations or a collection of local populations combined (Moran & Anderson, 2018). We first ran an analysis keeping all our source populations (Maria Is., Tinderbox, North Bruny Is., South Bruny Is., Partridge Is.) as reporting units, except for Southport where there were too few samples. North Bruny and South Bruny were separated based on results from STRUCTURE and DAPC and we kept Tinderbox and Partridge Is. separate from North Bruny and South Bruny, respectively, because we wished to quantify dispersal among these locations. We also conducted an analysis in which we combined individuals from Partridge Is. and South Bruny Is. into one reporting unit (i.e. South Bruny) given their proximity and results from STRUCTURE and DAPC.

Local effective population size (N_e)

We estimated local effective population size using the single-sample bias-corrected linkage disequilibrium method (LD; Waples 2006; Waples and Do 2010) implemented in the software NEESTIMATOR v2.1 (Do *et al.*, 2014). For this analysis we removed close relatives (i.e. full siblings) because their inclusion can inflate LD estimates leading to bias (Wang, 2018). Based on our previous analysis (see results), we combined individuals from South Bruny Is., Partridge Is.

and Southport into one group (i.e. South Bruny), and kept the other populations separated, i.e. Tinderbox, North Bruny Is. and Maria Is. Even though the LD method seems robust to certain levels of migration (Waples, 2010), for Tinderbox we removed six individuals that migrated from Bruny Is. identified in the assignment analysis as they can cause upward bias in local effective population size estimates (Luikart *et al.*, 2010). We also dealt with the structured populations by re-running the analysis combining Tinderbox and North Bruny Is. into one population (i.e. North Bruny), following the genetic clusters identified in the previous analyses (Luikart *et al.*, 2010). We conducted analyses under the assumptions of monogamy and random mating. We screened out rare alleles (which can create upward bias in LD estimates) with frequencies below two critical values (P_{crit} = 0.02 and 0.05; Turner *et al.* 2001; Waples and Do 2010), and calculated confidence intervals using the jackknife method (Waples and Do 2008; Do *et al.* 2014).

Genetic conservation prioritization

We conducted two analyses to inform management strategies following von Takach *et al.* (2021). We first followed Petit *et al.* (1998) and assessed the contribution of each population to the total allelic richness represented in the 4,368 SNP dataset (across all populations sampled). Based on our population structure analysis (see results), we first combined Partridge Is. and South Bruny Is. into one population (i.e. South Bruny) and removed individuals from Southport due to small sample size (n=3). We used the function *allel.rich* in *PopGenReport* package (Adamack & Gruber, 2014) and estimated mean allelic richness per locus for a sample of 50 alleles over the entire dataset (equivalent to the smallest sample size at Tinderbox). Then, we iteratively removed one population at a time from the dataset to estimate the proportional loss of allelic richness that would result if any one of these populations went extinct, using the formula AR(t) - AR(-i) / (AR(t) - 1). Where AR(t) is total allelic richness and AR(-i) is allelic richness over all populations excluding the one in question (Petit, Mousadik, & Pons, 1998).

We also used MARXAN software, which uses stochastic optimization routines to solve a range of conservation prioritization problems (Ball, Possingham, & Watts, 2011). This method identifies networks of extant populations that best represent the total genetic diversity in the species, as estimated by the sampled populations across the 4,368 SNP loci. For this exercise, we did not have a specific cost for the conservation options, so we allocated an equal unit cost for conservation of each population. Using the function *marxan_problem* in *prioritizr* package v 5.0.2 (Hanson *et al.* 2020) and the *Gurobi* solver (Gurobi Optimization and LLC 2020), we identified the optimal network of populations to maximise allelic richness in the species. We identified optimal solutions for scenarios of 1, 2, 3 or 4 'protected' populations, meaning that in scenario 1 for example, if we were to conserve only one population which one would best represent genetic diversity across populations. We also calculated the proportion of total alleles represented in each solution.

Results

Exploratory analysis

Summary statistics of genetic diversity metrics are presented in Table 1. Overall observed (H_o) and mean expected (H_s) heterozygosity were estimated at 0.19 and 0.20, respectively. H_o values were similar across populations, except for the more isolated population on Maria Is., which had lower H_o and H_e (expected heterozygosity) (Table 2). Mean F_{ST} (inbreeding coefficient) was 0.09 (pairwise values are presented in Table 3) and F_{IS} (population-level inbreeding coefficient) was 0.06.

Table 1. Mean and standard deviation of genetic diversity metrics.

Nº loci	Mean H_s	Mean H_o	Mean H_t	Mean F_{IS}	Mean F_{ST}	Mean call rate	Mean depth	Mean allele ratio	Mean repeatability

4,368	0.19 ± 0.14	0.19 ± 0.15	0.22 ± 0.16	0.05 ± 0.17	0.07 ± 0.50	0.99 ± 0.007	29.4 ± 17.7	1.58 ± 0.51	1 ± 0

Table 2. Estimated observed (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{IS}) for each geographic area sampled over a total of 4,368 variable SNPs across sites.

Location	**Sample size**	**Number of polymorphic loci**	**H_o**	**H_e**	**F_{IS}**
Maria Is.	79	3628	0.14	0.15	0.06
North Bruny Is.	172	3121	0.20	0.22	0.08
Partridge Is.	12	4165	0.19	0.20	0.06
South Bruny Is.	25	3850	0.19	0.20	0.07
Southport	3	4282	0.19	0.17	0.01
Tinderbox	25	3950	0.20	0.21	0.07

Table 3. Pairwise estimates of genetic differentiation (F_{ST}), between forty-spotted pardalote populations. All *p* values are < 0.01.

	Maria Is.	**North Bruny Is.**	**Partridge Is.**	**Southport**	**South Bruny Is.**
Maria Is.					
North Bruny Is.	0.19				
Partridge Is.	0.26	0.06			
Southport	0.27	0.05	0.01		
South Bruny Is.	0.24	0.06	0.03	0.02	
Tinderbox	0.24	0.03	0.07	0.06	0.07

Population genetic structure

The first two PCoA axes explained 16% of variation in genetic distances among individuals and grouped the genotypes into three clusters (Fig.2). The first PCoA axis separated the more isolated Maria Island population from the remaining sampled regions, while the second PCoA axis shows two clusters, one with individuals from North Bruny Is. and Tinderbox Peninsula, and a cluster with individuals from South Bruny Is., Partridge Is. and Southport (Fig. 2).

Discriminant analysis of principal components (DAPC) showed evidence of population structure with three clusters (K = 3), but also showed some support for a K = 4 model (Fig.3). The fourth cluster corresponded to a group of individuals in a very well-sampled region within North Bruny Is. (where we sampled many nestlings) and is likely driven by sampling of siblings. Given the similar levels of BIC support for K = 3 and K = 4 models, and the likely influence of sampling intensity on the K = 4 model, we focus primarily on interpreting results from the K = 3 model. Considering three clusters, individuals from North Bruny Is. and Tinderbox Peninsula clustered together (mainland site separated by 1.4 km of water), while individuals from South Bruny Is., Partridge Is. and Southport formed another cluster (Partridge is isolated by 0.4 km of water west of South Bruny, and Southport is a mainland site 4.4 km of water west of South Bruny). Maria Is. individuals were grouped into their own separate cluster (Fig.3).

Results from STRUCTURE (Pritchard, Stephens, & Donnelly, 2000) showed the same pattern of population structure to those suggested by the DAPC. STRUCTURE revealed K = 3 as the most likely number of genetic clusters (Fig. S5) for the model with correlated allele frequency among populations (Fig. 4), and K = 4 for the model with uncorrelated allele frequency (Fig. S6 and S7).

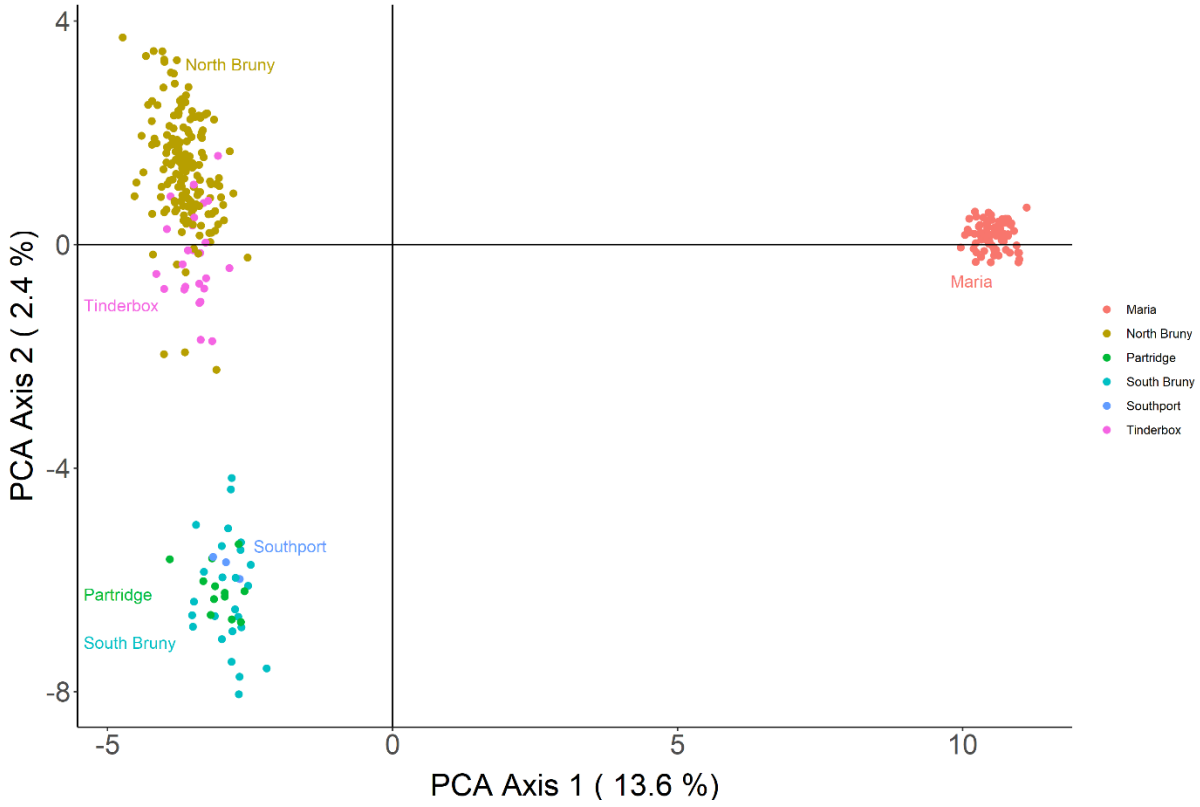

Figure 2. Principal coordinates analysis of individual forty-spotted pardalote SNP genotypes, coloured by sampling location.

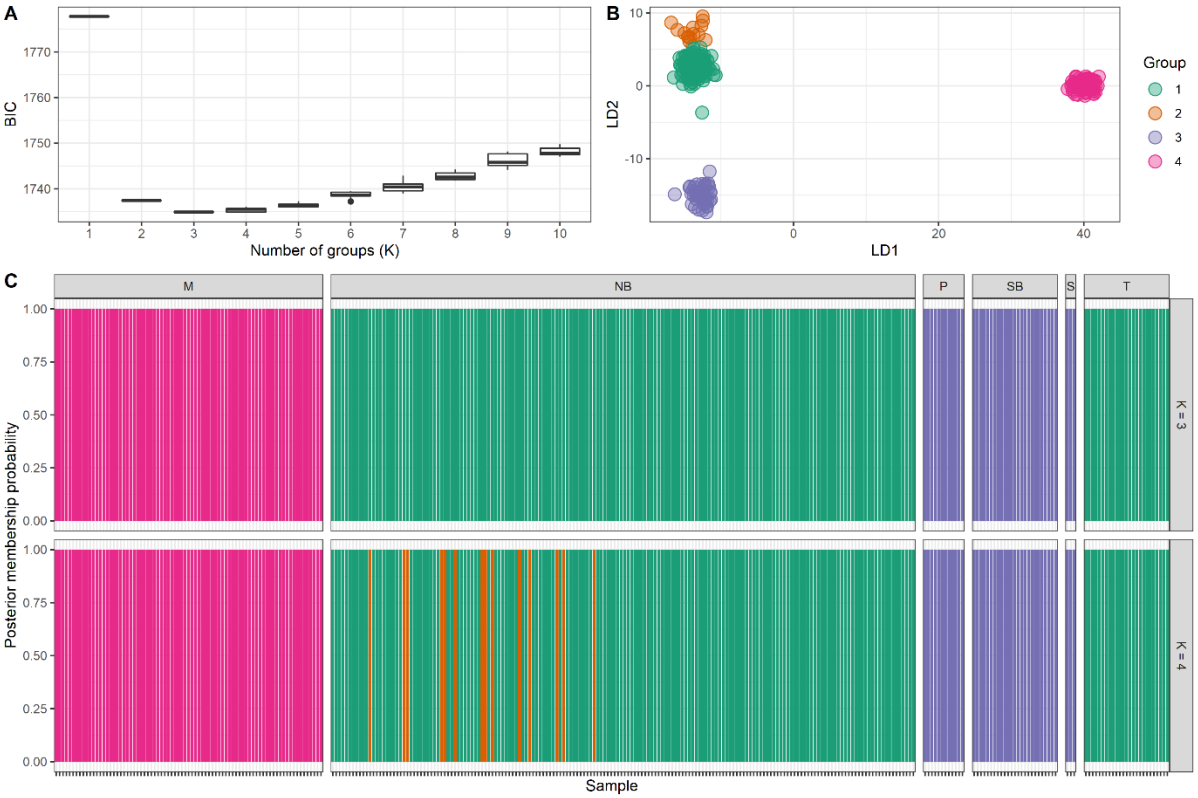

Figure 3. Discriminant analysis of principle components (DAPC). **A**) Values of BIC versus number of clusters (K); **B**) Scatterplot based on the discriminant functions showing four clusters (K = 4); **C**) Bar plots of the posterior probabilities of group assignment for each sample across K explored (each bar represents an individual). Header on plot **C**: M = Maria Is., NB = North Bruny Is., P = Partridge Is., SB = South Bruny Is., S = Southport, T = Tinderbox Peninsula.

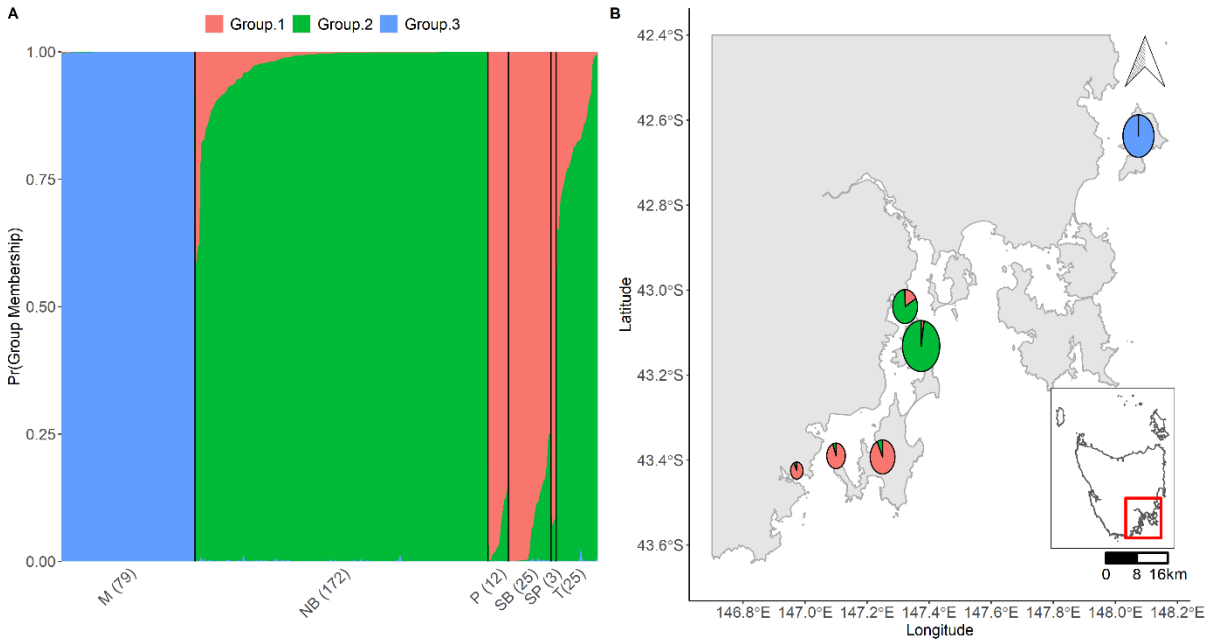

Figure 4. Results of STRUCTURE analysis with correlated allele frequency. **A**) Distribution of the probability of group membership for individuals in each population. Numbers in brackets represent sample size and plot labels are coded as: M = Maria Is., NB = North Bruny Is., P = Partridge Is., SB = South Bruny Is., S = Southport, T = Tinderbox. **B**) Each pie chart represents a sampled population (pie chart size reflects sample size shown in plot A), and colours the three clusters identified in the STRUCTURE analysis. An interactive plot with individual pie charts and membership probability can be found in supplementary material (Fig. S8).

Identification of immigrants

In the first run of the analysis in which we kept our geographically sampled populations as reporting units, most individuals were assigned to their reporting unit of origin. Six individuals from Tinderbox were assigned to North Bruny, corresponding to a 24% immigration rate into Tinderbox. All three individuals from Southport were assigned to either Partridge Island (n= 2) or South Bruny (n = 1; Fig. 5), but note that we did not include Southport itself as a candidate source population as only three individuals were found at that site. In the analysis where Partridge Is. and South Bruny were merged into one reporting unit, all individuals from Southport were

assigned to the merged unit. All individuals were assigned to the reporting units with probabilities near 1 and therefore were all accepted with high confidence (Table S1).

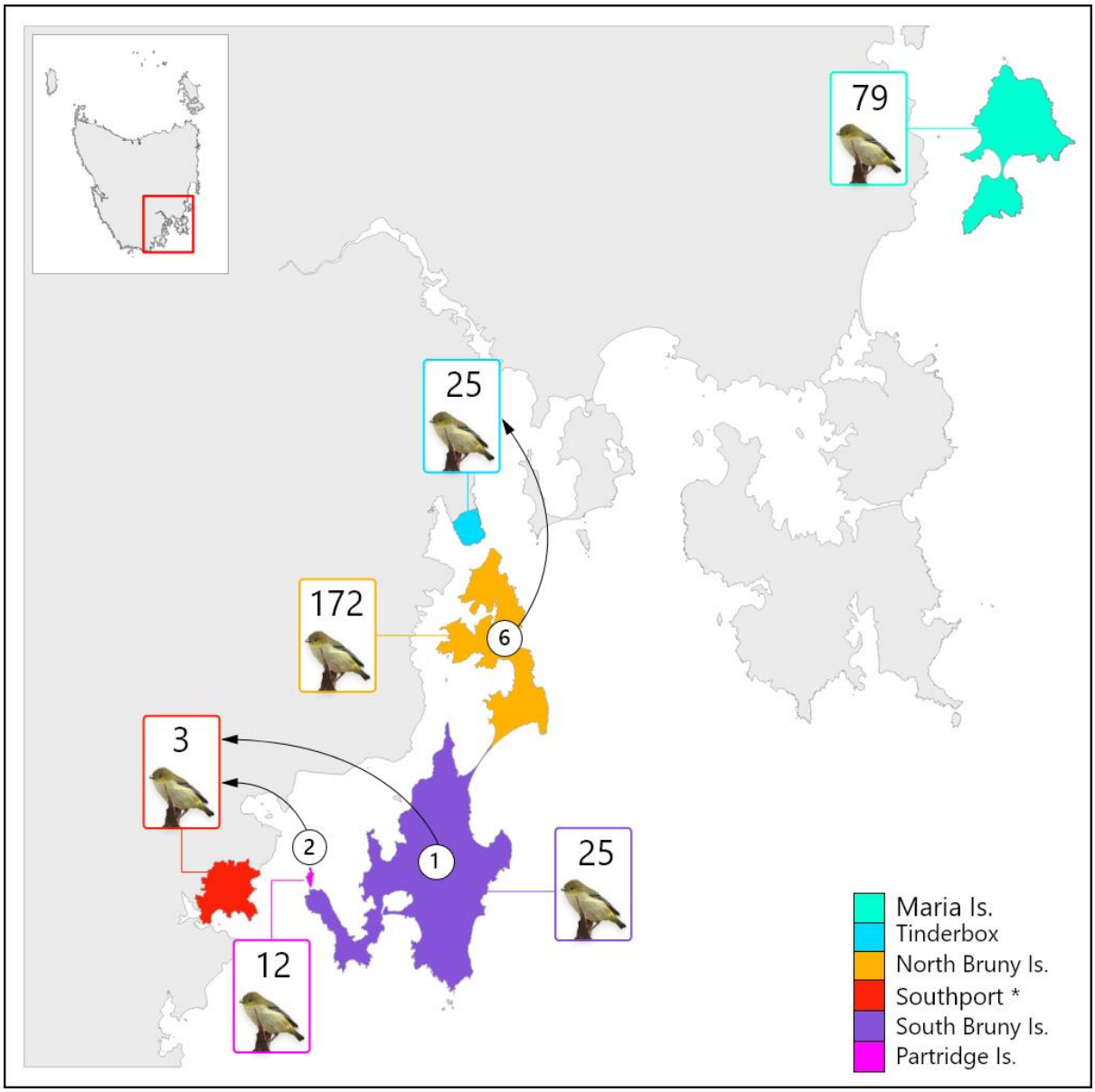

Figure 5. Results of the individual assignment analysis. Except for Southport*, all collection sites were used as reporting units (coloured areas). Boxes show sample size in each reporting unit and arrows indicate dispersal direction with the number of assigned dispersers.

Local effective population size (N_e)

Estimates of effective population size ($\hat{N}_e$) were similar across the two critical values used to screen out rare alleles (0.05 and 0.02; table 3). Maria Island had the largest effective population size ($\hat{N}_e$ = 629.7), followed by North Bruny Is. ($\hat{N}_e$ = 351.5), South Bruny Is. ($\hat{N}_e$ = 367.4) and Tinderbox ($\hat{N}_e$ = 67.9).

Table 3. Local estimates of effective population size ($\hat{N}_e$) with 95% confidence intervals in brackets for forty-spotted pardalote populations using the linkage disequilibrium method. Estimates are presented for both mating systems and for the two critical values used to screen out rare alleles.

	Random mating		**Monogamy**	
	P_{Crit} = 0.05	P_{Crit} = 0.02	P_{Crit} = 0.05	P_{Crit} = 0.02
Maria Is. (n=79)	318.6 (203.2-540.7)	334.8 (235-797.5)	629.7 (415.8-1167.8)	688.5 (518.2-2110)
North Bruny Is. (n=114)	167.2 (124.4-196.7)	189.9 (124.5-184.1)	351.5 (322.7-586)	349.1(347.1-668.8)
South Bruny Is. (n=31)	188 (91.6-366.4)	199.3 (∞)	367.4 (150.6-407)	415.7 (∞)
Tinderbox (n=19)	31.1 (16.3-51.9)	39.1 (17.8-44.9)	67.9 (45.8-264.2)	80.1 (38.8-99.6)
North Bruny Is. + Tinderbox (combined n=139)	162.2 (145.9-249)	176.6 (164.6-277.5)	340.1 (324.6-575.9)	349.3 (295.4-468.3)

Genetic conservation prioritization

North Bruny Is. has the highest allelic richness, followed by Tinderbox Peninsula, South Bruny Is. and Maria Is. (Table 4). However, in terms of contribution to total allelic richness across the species, the populations that contributed the most were North Bruny and Maria Island. The MARXAN results were similar and identified North Bruny as the single population that contains the highest proportion of the total genetic diversity in the species, and this was the priority population in a scenario where only a single population could be protected. Protecting North Bruny alone represented 97% of the alleles detected in the SNP panel we analysed. The second scenario identified Maria Is. as the next priority and South Bruny as the third priority (Table 4).

Table 4. Mean allelic richness with standard deviation for each population of forty-spotted pardalotes and allelic richness contribution following (Petit, Mousadik, & Pons, 1998). MARXAN results, showing population identified as priority in each scenario (solutions 1–4 represent alternate management strategies in which 1, 2, 3, or 4 populations can be protected with available resources), with proportion of alleles represented in each solution.

Population	Mean allelic richness	Allelic richness contribution	Solution 1	Solution 2	Solution 3	Solution 4
Maria Is.	1.51 ± 0.47	0.045	0	1	1	1
North Bruny Is.	1.83 ± 0.31	0.076	1	1	1	1
South Bruny Is.	1.73 ± 0.42	0.013	0	0	1	1
Tinderbox	1.77 ± 0.39	0.005	0	0	0	1
		Proportion of alleles	0.97	0.99	0.99	1

Discussion

We demonstrate the utility of investigating genetic parameters using the endangered forty-spotted pardalote and reveal previously undetected population genetic structure corresponding to geographical barriers and fragmentation. Based on our results, North Bruny Is. and Maria Is. are most important for representing the genetic diversity in forty-spotted pardalotes and should be prioritized for conservation. We also demonstrate connectivity among the southern populations via migration events and evidence of small effective population size, showing that some populations are vulnerable to genetic stochasticity (Frankham *et al.*, 2010). Below, we discuss our results in detail, and show that a genetic assessment can be highly informative for setting conservation goals for threatened species.

Genetic diversity

Our panel of 4,368 SNPs shows similar genetic diversity and low genetic differentiation across Bruny Is. and adjacent populations (H_o range: 0.19-020; F_{ST} range: 0.01 – 0.07), and even though the more isolated Maria Is. (74km NE of the closest mainland population) had lower genetic diversity (H_o=0.14) and higher divergence (F_{ST} range: 0.19-0.27), there is no evidence of inbreeding (F_{IS} = 0.06). Unlike some endangered species with fragmented populations and highly structured genetic diversity that require genetic restoration (e.g. Reynolds *et al.* 1999; Westemeier *et al.* 1998; Mitrovski *et al.* 2007, Weeks *et al.* 2017; von Takach *et al.*, 2021), genetic diversity of forty-spotted pardalotes is represented in most remaining populations, with no immediate need for genetic management. Higher genetic differentiation on Maria Is. reflects the lack of gene flow from other remaining populations. Similar patterns have been described for other passerine species, where higher divergence as a result of limited dispersal due to fragmentation and unsuitable habitat was reported even at smaller spatial scales than seen in our system (e.g. Callens *et al.* 2011; De Camargo *et al.* 2015).

Population structure and management units

We identified management units from genetic data in the context of two questions. The first relates to local recruitment dynamics and whether a number of small mainland Tasmanian populations should be considered demographically discrete units for management or whether they are dependent on other areas for recruitment. The second question relates to how best conserve genetic diversity in the species and we addressed this by identifying discrete populations that can be considered discrete units in terms of their contribution to total genetic diversity in the species and for planning potential genetic management within and among those units. The foundational information for both of these management questions was the analysis of genetic structure and the conservation prioritization analyses.

We considered a model of three genetic clusters most relevant to conservation planning. This model showed genetic structure even within Bruny Is., where two clusters (north and south Bruny) connected to their nearby mainland sites were detected. Migrants from Bruny Is. detected on mainland sites further confirmed connectivity between southern populations. This stronger connectivity between Bruny Is. and adjacent mainland populations rather than between the north and south portion of the island may be explained by unsuitable habitat. Forty-spotted pardalote habitat (i.e. white gum forest) on North Bruny Is. is highly fragmented, and the isthmus connecting north to south is long and covered by heath (~7 km of unsuitable habitat). Therefore, the genetic structure within the island is likely to be driven by unsuitable habitat and potentially compounded by fragmentation of suitable habitat to both the north and south of the isthmus (e.g. Peery *et al.* 2010; Adams and Burg 2015; Schlaepfer *et al.* 2018), making dispersal to adjacent mainland sites easier. Although Maria Is. also has an isthmus, it is much smaller (700 m) and mostly covered by forest (including white gums), allowing dispersal between north and south, which is evident in the single genetic cluster we detected on the island.

Both analyses used in our conservation prioritisation approach identified North Bruny and Maria Is. as most important for representing the genetic diversity in forty-spotted pardalotes, with North Bruny alone contributing 97% to total allelic richness. When North Bruny Is. and Maria Is. are combined total richness rises to 99%. Although we do not have data to suggest these populations fulfil the requirements of evolutionarily significant units, they are important distinctive units, and therefore we consider Maria Is.

and the southern populations as different units (i.e. 2 management units) for the purposes of conserving genetic diversity in the species as they reflect population units with little migrant interchange. For managing populations and facilitating species recovery, we use the traditional definition of management units (i.e. considering demographic processes). We identify three units based on the structure and migration patterns we found on the southern populations with Tinderbox and Southport clustered with North Bruny Is. and South Bruny Is., respectively as they are dependent on these for recruitment. The connectivity among the southern populations indicates that forty-spotted pardalotes have some capacity for cross-water colonisation of suitable habitat, which is useful for a restoration perspective. If genetic management of forty-spotted pardalotes is considered in the future, translocations between the North Bruny/South Bruny Is. and Maria units would result in greatest increase in genetic diversity, although at this stage the relative gain in allelic diversity from such actions is low.

Local effective population size

Mating system has a major influence on effective population size (Richard Frankham, Ballou, & Briscoe, 2010), and although we estimate effective population size for both random mating and monogamy, because pardalotes are socially monogamous, we concentrate on interpreting the results for monogamy. However, it is likely that their true effective population size is somewhere between the two mating systems, given that genetic monogamy (i.e. lifetime pair bond) is rare in passerines (Hasselquist & Sherman, 2001).

Effective population sizes were small for Bruny Is. and Tinderbox Peninsula (North Bruny Is. = 351.5, South Bruny Is. = 367.4, Tinderbox = 67.90) in comparison to Maria Island ($\hat{N}e$ = 629.7). These results may seem surprising, given that genetic diversity on the southern populations was higher than Maria Is., and populations with smaller effective population sizes are expected to have decreased genetic diversity due to the effects of inbreeding and drift (Frankham 2005). However, the pattern we found here may be explained by a combination of different habitat quality between the southern populations and Maria Is., and the effect of population structure on linkage disequilibrium. Recent land-clearing and fragmentation

on Bruny Is. has potentially affected population connectivity leading to the structure we found in the southern populations. Given that population structure can affect patterns of linkage disequilibrium (e.g. Maccaferri *et al.*, 2005), which is the method we used to estimate Ne, any recent changes in population size would likely be observed in linkage disequilibrium patterns before changes in heterozygosity. The fragmented landscape in which the southern populations occur in combination with other known threatening processes, such as low nesting site availability and reduced breeding success (Edworthy *et al.* 2019; Alves *et al.* 2020) likely contributed to the smaller contemporary local population size estimated for these populations. Although Maria Is. is isolated and has lower genetic diversity, the island is a national park dominated by contiguous, mature native forest. This better habitat quality probably allows increased reproductive success and the maintenance of higher effective population size in local forty-spotted pardalotes (e.g. Reed 2004).

Even if the mating system of pardalotes is genetically monogamous (for which local N̂e values were higher), effective population sizes for the southern populations are below those required to maintain sufficient evolutionary potential in the long term (Ne > 500) as predicted by Franklin and Frankham (1998). Some argue that the Ne > 500 rule should in fact be doubled for maintaining evolutionary potential in perpetuity (Richard Frankham, Bradshaw, & Brook, 2014), in which case all populations are below this threshold. Further, if their mating system is non-monogamous, Tinderbox (N̂e = 31.1) is already below the size needed to minimise short term problems (Ne = 50, Franklin and Frankham 1998; Ne > 100, Frankham, Bradshaw, & Brook, 2014), indicating that some populations are vulnerable to genetic stochasticity (Richard Frankham, Ballou, & Briscoe, 2010), and showing the necessity to implement actions that increase populations size.

Informing the conservation of genetic diversity

Our results show that implementing effective habitat management on Bruny Is. and the adjacent Tasmanian mainland should be a priority. The populations in these areas are vulnerable to genetic stochasticity due to their small local population size and the presence of known threats such as habitat

loss driven by land clearing and tree dieback. Most of the remaining populations are in areas of disturbed, young forest where nest site availability is low, leading to increased competition (A. Edworthy, 2016a). Further, parasitism from fly larvae on nestlings has been detected as a new threat, severely reducing recruitment (at least on North Bruny Is.; Edworthy *et al.* 2019). In addition, although our results do not allow us to infer the demographic effects of migrants, the asymmetric migration we detected (i.e. one-way migration off Bruny Is.), suggests the mainland sites may be sink habitats (Pulliam 1988; Gaggiotti 1996), particularly Southport where we sampled only three individuals. Forty-spotted pardalotes were rediscovered in Southport in 2015 after having not been recorded at this site for >120 years (Webb *et al.* 2019). Our small Southport sample size reflects the low density of the species in that area. In fact, all three individuals were migrants from Bruny and Partridge Is, and therefore it is likely that migrants are constrained by limiting factors that impede population expansion within Southport. Nonetheless, these dispersal events are encouraging and highlight the importance of Bruny Is. for maintaining adjacent populations, and the potential for habitat and threat management to lead to population expansion via colonisation. For Southport, provided there is enough area and habitat restoration is undertaken to address limiting factors, translocating individuals from South Bruny Is. and Partridge Is. might be an effective strategy if migration rates are too low. For Tinderbox, habitat restoration may be sufficient to maintain the population, as we detected a 24% immigration rate into Tinderbox.

Considerations for using translocation as a conservation tool

Conservation translocation has been proposed for forty-spotted pardalotes to create insurance populations (Webb *et al.*, 2019) and our genetic assessment can be used to inform translocations that consider genetic diversity (e.g. Weeks *et al.* 2015). Given that North Bruny Is. and Maria Is. hold most genetic diversity in the species, these populations could be used to source founders following the 'genetic-capture' approach for threatened species described by Weeks *et al.* (2011, 2015). Sourcing founders from both populations should be considered to increase genetic diversity (Weeks *et al.*, 2011) and reduce the demographic impact on the source populations. This is an important consideration

because intensive collection of founders can negatively impact the remaining source population for years after collection occurs (e.g. Morrison *et al.* 2020). Although mixing populations for genetic rescue effect has often been avoided due to fears of outbreeding depression (i.e. loss of fitness when genetically different populations are crossed), the risk is generally low and often predictable (Frankham *et al.* 2011; Frankham *et al.* 2019). Moreover, outbreeding depression primarily occurs due to adaptive differentiation (Richard Frankham *et al.*, 2011), which is not the case in most threatened species where differentiation is likely to be driven by fragmentation followed by interrupted gene flow, resulting in small effective population size and drift (Reed and Frankham 2003; Lowe *et al.* 2005; Coleman *et al.* 2013; Weeks *et al.* 2015). There is also growing empirical evidence that genetic rescue can increase genetic diversity and population viability (Frankham 2015; Whiteley *et al.* 2015; Kronenberger *et al.* 2017; Ralls *et al.* 2018; Fitzpatrick *et al.* 2020).

While our study is important for informing discussion about the feasibility and need for translocations, there remain multiple unresolved conservation problems. Forty-spotted pardalotes are habitat specialists, so habitat assessment and management should be undertaken prior to any translocation because unoccupied habitat is not necessarily suitable habitat (Osborne & Seddon, 2012). Failing to remove or manage known threats will lead to failure even if the best genetic strategy is used to inform translocation. More importantly, if there is insufficient funding, we argue that conservation efforts should focus on managing the southern populations to increase their effective population sizes by expanding the availability of suitable habitat, before attempting translocations. Likewise, even though we did not consider the Flinders Island population in this study, our results suggest that this subpopulation is likely to constitute its own conservation management unit. However, little is known of the status of pardalotes on Flinders Island and confirming whether they (i) still survive and (ii) are viable, should be a high priority. If the Flinders Island population is extant but genetically diminished, it may be a good candidate for habitat restoration and translocation from other populations.

Conclusion

Our study has important implications for the management of forty-spotted pardalotes and shows that by assessing contemporary genetic aspects, valuable information for conservation planning and decision-making can be produced to guide actions that account for genetic diversity. Incorporating genetic management alongside other management actions is crucial to increase the chances of species recovery. The compounding effects of environmental, demographic and harmful genetic processes can create an extinction vortex (Gilpin & Soulé, 1986), and failing to account for the genetic problems may undo other good conservation actions. The neglect of genetic factors in species management needs to change (Ralls *et al.*, 2018) because most species are not driven to extinction before the impact of harmful genetic processes is manifested (Spielman, Brook, & Frankham, 2004). Moreover, climate change will bring new challenges to species management, and genetically diverse populations have a greater potential to adapt (e.g. Bitter *et al.* 2019). Our study reinforces the need to consider population genetic tools as fundamental to the practice of conservation biology. Using this type of information enriches knowledge of the demographic processes that shape small populations, and provide managers with defensible information that they can use to optimise their conservation strategies.

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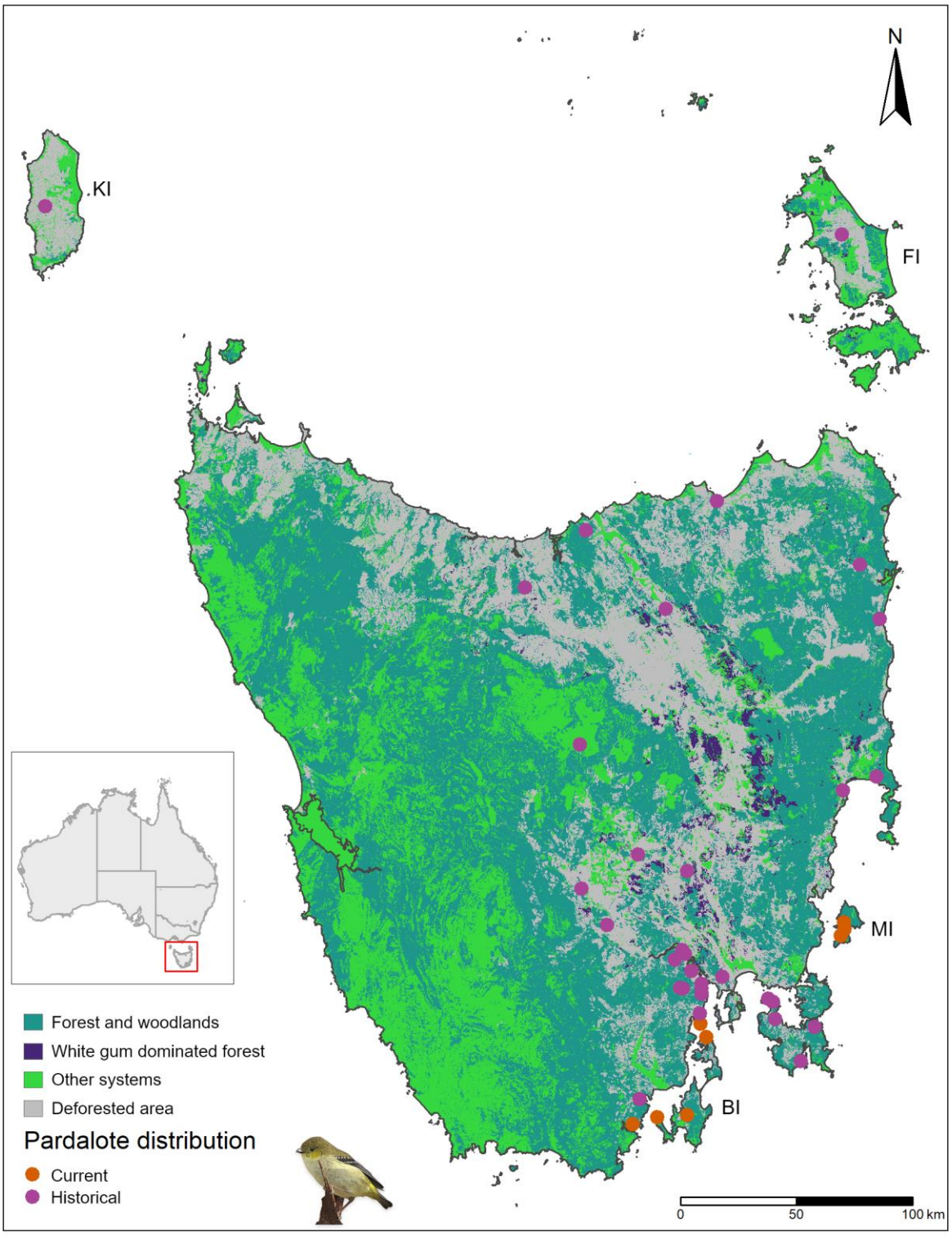

Figure S1. Current and known historical distribution of forty-spotted pardalote across Tasmania, showing mapped white gum dominated forest (pardalote's preferred food tree) within remaining forest cover according to TASVEG 4.0 (Department of Primary Industries, Parks, Water and Environment, 2020). Offshore islands: KI = King Island, FI = Flinders Island, MI = Maria Island, BI = Bruny Island.

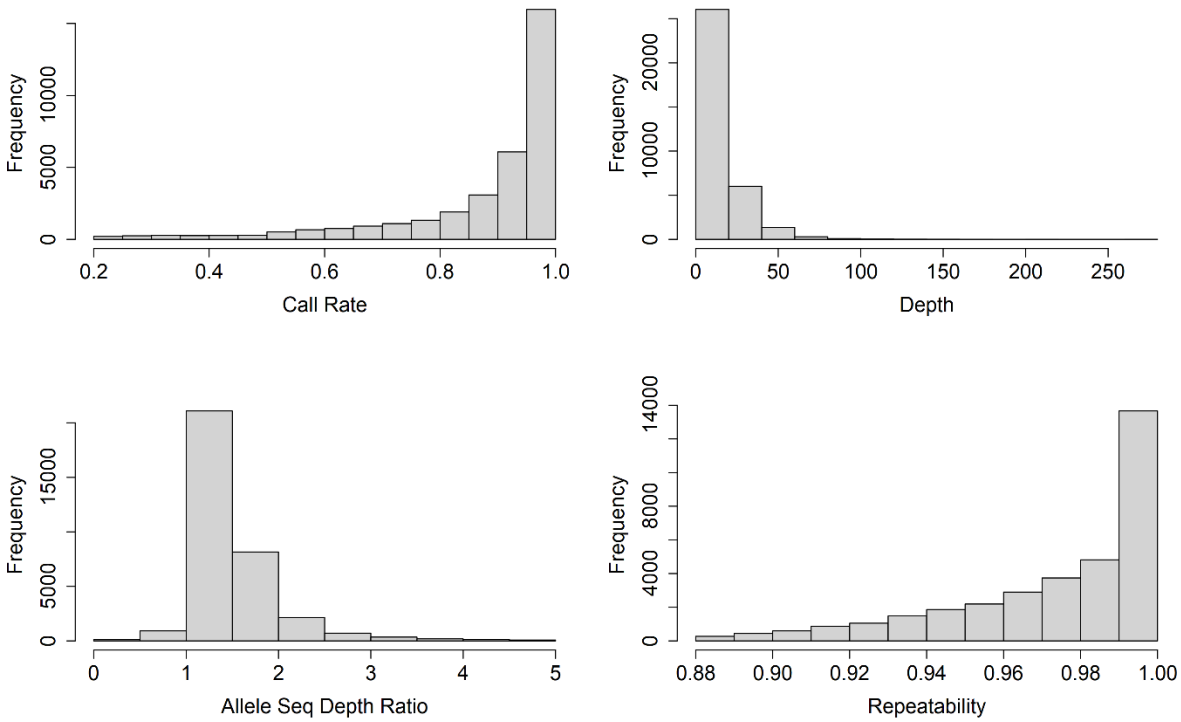

Figure S2. SNP metrics pre filtering

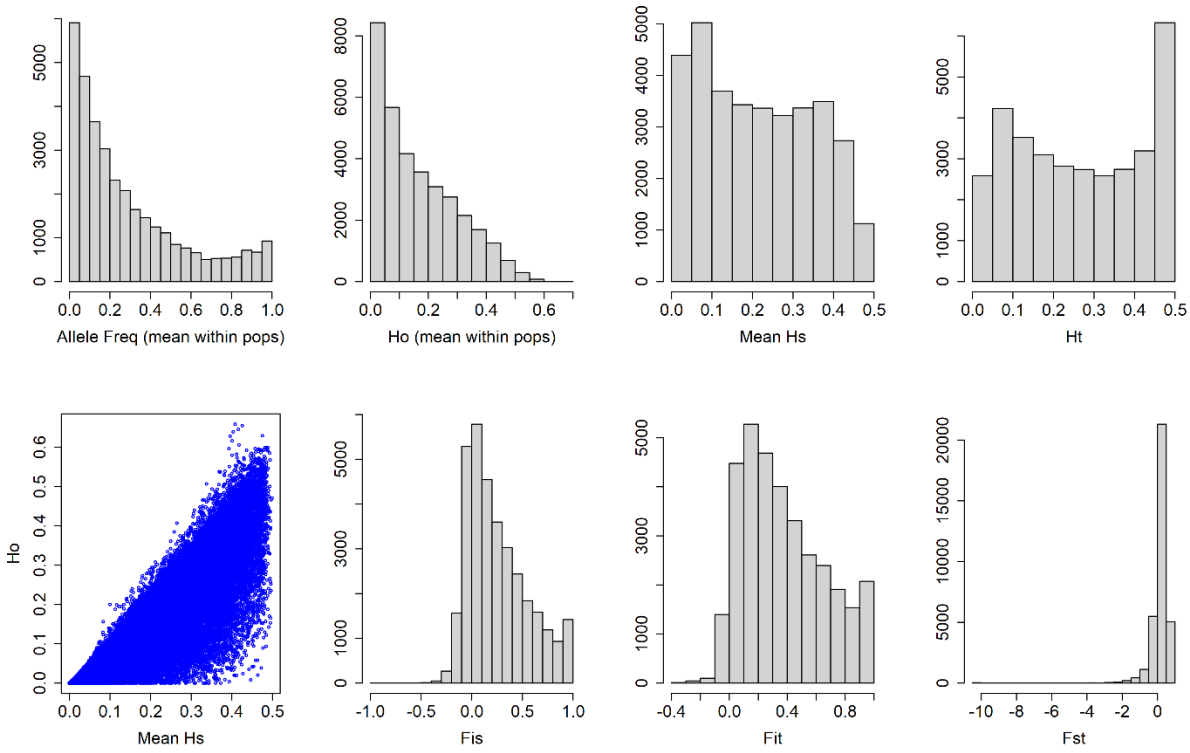

Figure S3. FST pre filtering

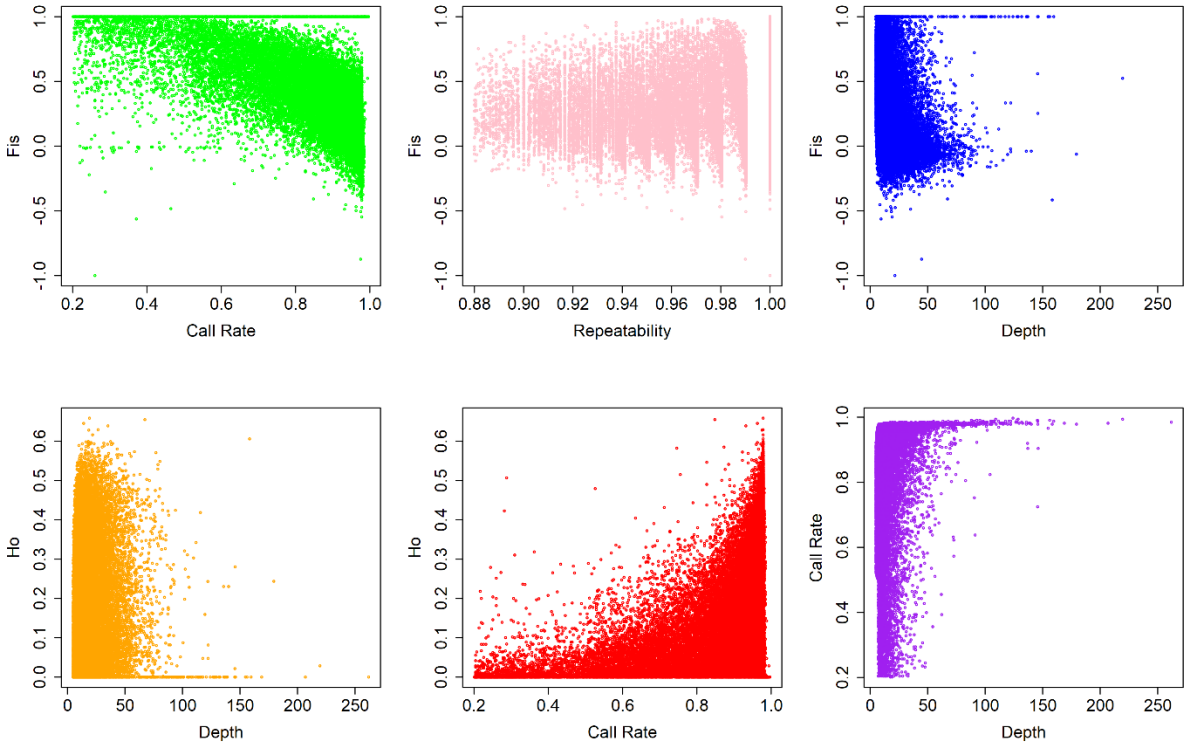

Figure S4. Population genetic diversity statistics against SNP quality pre filtering.

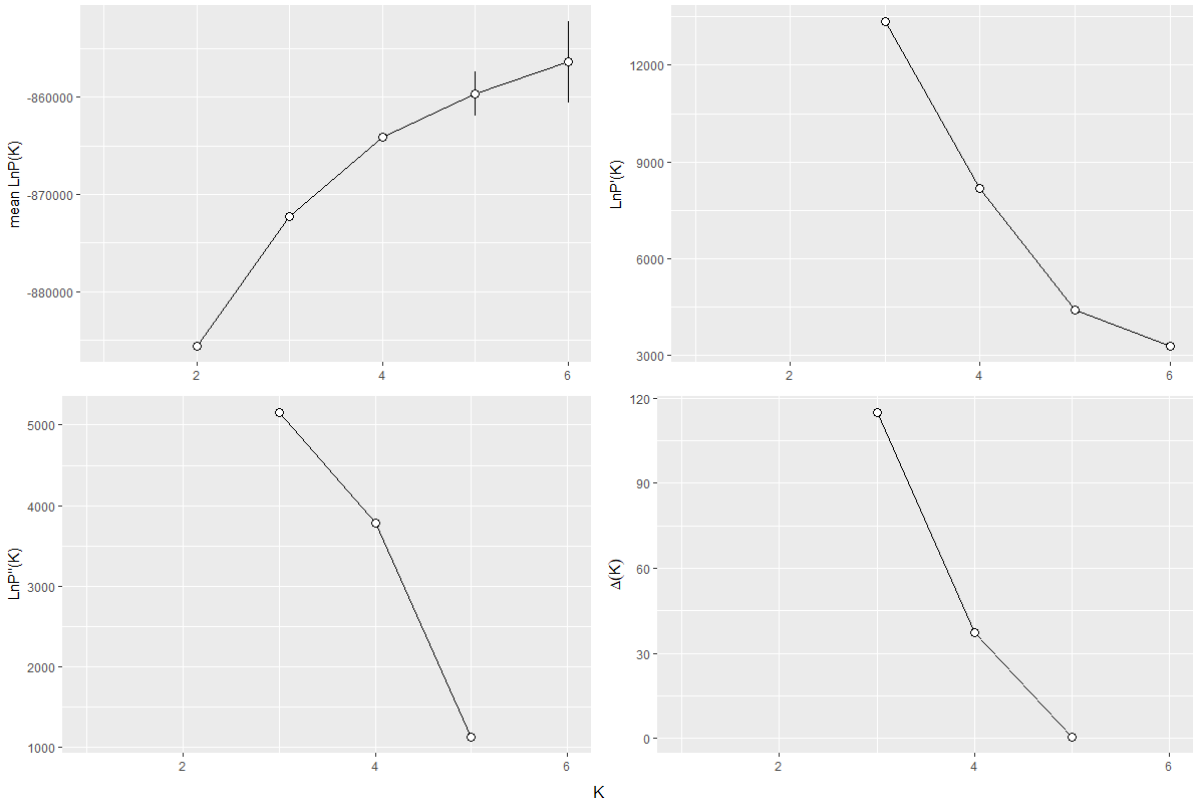

Figure S5. Diagnostic plots of the model with correlated allele frequency. First and second order changes in the likelihood for K (number of groups) for STRUCTURE analysis following Evanno (2005).

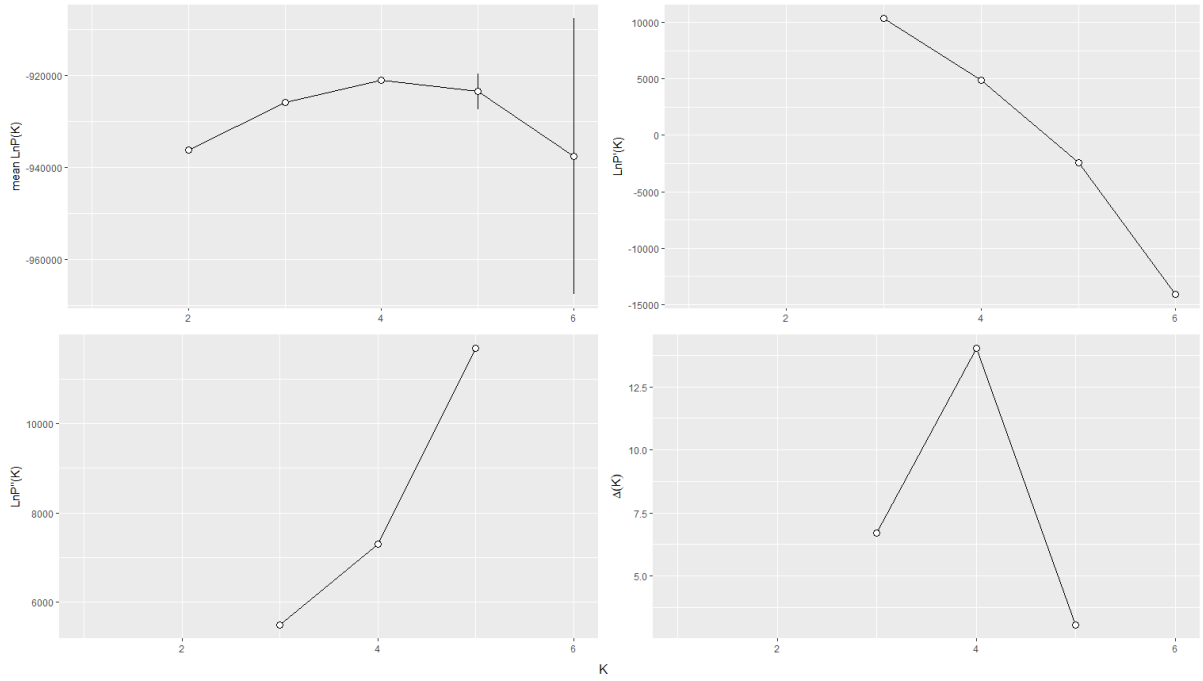

Figure S6. Diagnostic plots of the model with uncorrelated allele frequency. First and second order changes in the likelihood for K (number of groups) for STRUCTURE analysis following Evanno (2005).

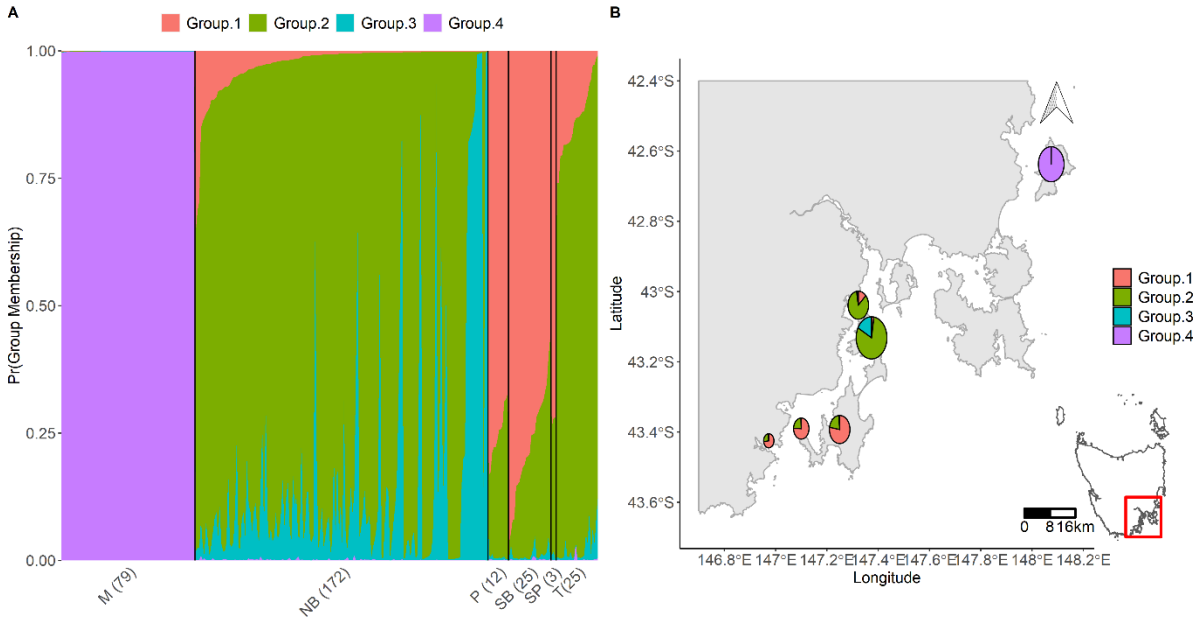

Figure S7. Results of STRUCTURE analysis with uncorrelated allele frequency. **A**) Distribution of the probability of group membership for individuals in each population. Number in brackets represent sample size and plot labels: M = Maria Island, NB = North Bruny Island, P = Partridge Island, SB = South Bruny Island, S = Southport, T = Tinderbox. **B**) Each pie chart represent a sampled population, and colours the

three clusters identified in the STRUCTURE analysis. The fourth group identified is a result of a highly sampled region (related individuals).

Figure S8. Interactive map with individual pie charts and membership probability for the STRUCTURE analysis with correlated allele frequency. Interactive map can be accessed at https://osf.io/anztp/.

Table S1. CSV file showing the result of the individual assignment analysis. File can be accessed at https://osf.io/h4zyc/.