Conservation genomics of the endangered Seychelles Magpie-Robin (*Copsychus sechellarum*): a unique insight into the history of a precious endemic bird

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The Seychelles Magpie-Robin *Copsychus sechellarum* is an IUCN Red-List Endangered species endemic to the Seychelles, whose population was reduced to eight individuals on a single island in the 1960s. Translocations from the remaining population to four additional islands have been an integral factor in their recovery, but the potential genetic consequences of their translocation history have not previously been explored. We resequenced the genomes of 141 individuals sampled across the five current island populations and analysed the data to characterize their population structure, as well as to explore suspected inbreeding. Overall, very low levels of heterozygosity were observed, all coupled with long homozygous segments that suggest recent inbreeding, probably the consequence of a population bottleneck in the 1960s. Three of the four translocated populations displayed less genetic diversity than the founder population from which they were taken, a familiar pattern observed as a result of the evolutionary force of genetic drift following founder events. Furthermore, and perhaps surprising given the recent time since the new populations were established, population structure was observed within these same three populations. New awareness of inbreeding in the Seychelles Magpie-Robin populations, and continued genetic monitoring, will allow for genetically informed management decisions. This is particularly prudent in maximizing the success of the future conservation translocation planned for this species.

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Driven to near-extinction in the mid-1900s, the Seychelles Magpie-Robin *Copsychus sechellarum* is one of many species endemic to the Seychelles to have been devastatingly affected by human actions over past centuries (Government of Seychelles 2014). The decline of this bird species, now listed by IUCN as Endangered, was due to agricultural intensification and the introduction of exotic predators, namely rats and cats (Gaymer *et al.* 1969, Wilson & Wilson, 1978, Watson *et al.* 1992), and was exacerbated by continued specimen collection as the population dwindled (in Oustalet 1878, as highlighted in Burt *et al.* 2016). The population, which by 1965 had been reduced to only eight sighted individuals on a single island, Frégate, had failed to recover naturally by 1990, resulting in management intervention from Birdlife International and The Royal Society for the Protection of Birds (RSPB), through the implementation of a recovery plan. As a result of this successful intervention, IUCN down-listed the species conservation status in 2005 from Critically Endangered to Endangered, thus rendering the Seychelles Magpie-Robin one of only 32 bird species to be reclassified positively (down-listed) between 1988 and 2008 (Birdlife International 2008). However, this reclassification was performed without insight into the genetic consequences of the species recovery plan, so the recovery plan did not consider the genetic vulnerability of the species.

An integral component of the successful Seychelles Magpie-Robin recovery plan was facilitation of population expansion through translocations, which has elevated the population size to nearly 400 individuals today. However, the success of the independent translocations was extremely variable. Six translocation attempts were carried out from Frégate to Aride Island (1978: six birds; 1979: four birds; 1992: two birds; 1994: one bird; 1995: six birds; 1999: one bird); and one fertilized egg was translocated from Cousin to Aride Island in 2000, all of which failed to establish successful populations (Burt *et al.* 2016).

Other translocations were more successful, principally (though not exclusively) involving birds from Frégate Island (Fig. 1). Specifically, between 1994 and 1996, nine birds were translocated from Frégate to Cousin Island; between 1995 and 1996, seven birds were translocated to Cousine Island, six from Frégate complemented with a seventh from Cousin Island; in 2002, the two surviving Magpie-Robins on Aride were moved to Frégate Island, and 15 new birds were moved from Frégate to Aride Island; and in 2008, a total of 20 birds were translocated to Denis Island: 16 birds from Frégate and four from Cousin.

In addition to sourcing birds from different islands, different strategies were also employed when choosing birds to be moved. Specifically, the donor birds for the Cousin and Cousine translocation were targeted as unrelated (as far as knowingly possible) adult breeding pairs. In contrast, for Aride some social groups were moved together (Bristol *et al.* 2005). Later, and based on observations derived from monitoring the results of the previous translocations, the donor birds for Denis Island were targeted to account for age (to include a high number of sub-adults and other young adults) and reproductive history (including reproductive, hatching and fledging success), alongside the exclusion (where possible) of known relatives (The Seychelles Magpie-Robin Recovery Team (S.M.A.R.T.) pers. comm.).

Today, the species inhabits five islands in total. Of these five islands, Frégate Island (Pike 1872, Hartlaub 1877) and Aride Island (Hartlaub 1877, Oustalet 1878) are the only islands with a documented history of Magpie-Robin populations previous to the recent translocations. There is believed to be very limited gene flow between these five islands; despite intensive monitoring, only 16 cases of island-to-island dispersal have been recorded, of which only seven resulted in establishment (Burt *et al.* 2016, S.M.A.R.T. pers. comm.). If an individual survives to adulthood, the average lifespan is 4.2 years (Nature Seychelles unpubl. data) but Magpie-Robins have been documented to live for up to 15 years and have an estimated generation time of 3.6 years (Birdlife International 2017). A breeding pair may occupy a territory for substantially longer than this, living with up to eight non-breeding subordinates within the territory. Related subordinate Magpie-Robins have been observed to act as helpers in the group – feeding the chicks. However, the presence of subordinates is largely recognized as causing conflict within the

Figure 1. Seychelles Magpie-Robin conservation translocations 1994–2008: a cropped map of Seychelles demonstrating the relative positions of each of the Magpie-Robin islands. Directionality of translocations for donor-to-receiver is indicated by coloured arrows, along which the number of birds donated is defined in bold, and the year/s of translocation events is/are given in parentheses. Islands are labelled by number corresponding to the order of translocations, and have been assigned the colour of the island identifier ring used when banding, which correspond as follows: 1 (green) Frégate; 2 (red) Cousin; 3 (blue) Cousine; 4 (yellow) Aride; 5 (orange) Denis. The distance between Frégate and Denis is roughly 65 km; between Aride and Denis is 45 km; between Aride and Cousin is 10 km; and between Cousin and Cousine is 2 km. The Seychelles archipelago has a total landmass of 459 km^2. The total area inhabited by the Magpie-Robins is only 4.86 km^2.

territory; given the small islands and limited territories and resources, this can ultimately slow the process of recovery (López–Sepulcre *et al.* 2008).

For two decades following the first translocation carried out by Birdlife International and RSPB, the future of this species looked promising with continual population growth on all established islands (Fig. S1). However, in 2014, the population of Aride suffered a sudden and catastrophic crash, where the population was reduced to just six birds from a population of 30, with no apparent environmental cause.

This crash ignited recognition of the need for genetic research on this species. We sequenced the genomes of 141 individuals representing the extant populations of the five islands. Our study had three main aims: (1) to explore how the genetic architecture of the four translocated populations differs from the founder population of Frégate Island; (2) to describe whether these translocations may have had potentially problematic consequences on the populations' genomic diversity as a result of bottlenecks or inbreeding; and (3) if so, to identify whether there may have been underlying genetic components potentially responsible for the 2014 Aride Island population crash.

METHODS

Sampling

Blood samples are routinely collected from Magpie-Robins by trained local conservation staff on all five islands, as part of the long-term monitoring programme. Blood sampling is carried out by the withdrawal of blood (maximum 70 μL) through brachial venepuncture, the extracted blood is stored in 1 mL of either lysis buffer, Queen's buffer or ethanol, and samples are subsequently frozen at –20 °C. A total of 141 blood samples were used for this research: 118 were provided by S.M.A.R.T. and 23 by the Netherlands Institute of Ecology (NIOO). As a result of the small current population sizes of the species, this sampling covers a representative sample of the populations at the time of this research, spanning each of the five islands inhabited by the Magpie-Robins today: Frégate (31/145), Denis (32/78), Cousin (31/50), Cousine (17/50) and Aride (30 total: 6/10 of the surviving population and 24 samples from NIOO that were archived blood samples from birds recently deceased and representative of the Aride population collapse). Samples were exported with the express purposes of undertaking this research project with permission from the Ministry of Environment, Energy and Climate Change of the Seychelles (MTA-2017), and were imported to Denmark for research under permission from the Ministry of Environment and Food of Denmark (J.nr. 2017-12-711-06242).

Laboratory protocols

Two different genomic DNA extraction kits were used based on compatibility with sample storage buffers. Samples stored in lysis and Queen's buffers were extracted using the Thermo Scientific King-fisher Blood DNA kit (ThermoFisher Scientific Inc., Waltham, MA, USA) with the adjustment of using 200 μL of sample input. The Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) for nucleated blood was used for samples stored in ethanol. Extractions were quantified using the Qubit Fluorometer 2.0 Broad Range DNA assay kit (Invitrogen, Carlsbad, CA, USA) and DNA quality was assessed using the genomic DNA kit for the Agilent TapeStation 2200 (Agilent Technologies, Santa Clara, CA, USA).

DNA was sheared following the standard protocol of the Covaris M220 Focused–Ultrasonicator with the adaptation of using a treatment time of 175 s to produce 250 base-pair fragments. One hundred and forty-four BGISeq-compatible genomic shotgun libraries were built following the BEST 2.0 protocol (Carøe et al. 2017, as modified by Mak et al. 2017) with the adjustment of using double-volume reactions because DNA input was high (as high as 500 ng/μL for some samples). The libraries were single-indexed and polymerase chain reaction (PCR)-amplified using the recommended cycles ascertained by quantitative PCR to avoid excessive duplicates (8–18 PCR cycles), and final PCR-amplified libraries were quantified using the Qubit Fluorometer 2.0 and the High Sensitivity DNA assay kit. All genomic shotgun libraries were pooled equimolarly in batches of eight and submitted to BGI-Shenzen (Shenzen, China) for sequencing using the BGIseq500 platform (BGI 2019) and paired-end reads of 100 base pairs.

Data analysis

The PALEOMIX pipeline v1.2.12 (Schubert et al. 2014) was used to remove adapters (Adapter-Removal v2.2.2) and to remove PCR duplicates with java v1.8.0 (Lindgreen 2012) from the raw sequencing reads. Using BWA-mem (v0.7.15) (Li 2013), the resulting filtered reads for each individual were then mapped against the Seychelles Magpie-Robin reference genome (Feng et al. 2020), and indel-realigned BAM files were subsequently generated (Genome Assembly Tool Kit (GATK) v3.8.0).

Sex-linked scaffolds were removed to reduce bias introduced by differing sequencing coverage, mixed ploidy, local mutation rate and sex-derived diversity found in avian sex chromosomes (Fridolfsson & Ellegren 1999, Montell et al. 2001). Scaffolds were identified as autosomal (not sex-linked) following identification and isolation of Z- and W-linked scaffolds determined by coverage, where males have twice the coverage of females. Only autosomal scaffolds over 100 kb in size were retained, culminating in a total of 232 (of 3664) scaffolds (maximum genome size of 0.95 Gb for each individual) for ANGSD v0.921 (Korneliussen et al. 2014) genotype likelihood computation (-rf, region file). Additionally, locations of repetitive regions (paralogues with high levels of heterozygotes that could skew results) were provided by Feng et al. (2020) and were removed from the final scaffold list with bedtools v2.26.0 -subtract (Quinlan & Hall 2010) and from downstream analyses using the -rf option in ANGSD.

Given the heterogeneous sequencing coverage across the individuals (c. 2- to 57-fold coverage), and in particular the low coverage of a subset of the samples (Table S1), we used genotype likelihood-based methods to propagate the uncertainty in genotype calls to downstream analyses (Korneliussen et al. 2014). Using the alignments generated with the PALEOMIX pipeline, genotype likelihoods were computed with ANGSD (v0.921) using the samtools model (Li et al. 2009) for genotype likelihoods (-GL 1). Genome-wide single nucleotide polymorphisms (SNPs) were identified using the genotype likelihood-based analysis. In order to ensure a high-quality dataset, filtering parameters and quality thresholds were used as follows: minimum read quality and mapping qualities of 30 (-minQ 30, -minMapQ 30), filtering of excessive mismatches (-C 50), base alignment quality (-BAQ), minimum site depth of three times the average coverage of the population (-setMinDepth), a minimum minor allele frequency of 5% (-minMaf 0.05) and a minimum P value of 1e-6 for an SNP to be called (-SNP_pval 1e-6).

The genotype likelihoods from the 141 individuals were used to identify a total of 604 408 polymorphic sites, which were subsequently used to generate a pairwise covariance matrix with PCAngsd v0.982 (Meisner & Albrechtsen 2018). The pairwise covariance matrix was used as input for the factoextra package in R/3.4.3 (R Development Core Team 2020) to compute and visualize

the principal components of the genotype data, plotted with 95% confidence ellipses.

Using the same genotype likelihood data for all 141 individuals as input, NGSadmix (v32) (Skotte *et al.* 2013) was used to estimate ancestry proportions for all individuals for a given number of ancestry components. Up to five ancestral components were estimated (*k* ranging from 2 to 5) with up to a maximum of 250 iterations to obtain the highest likelihood estimate.

Genetic differentiation between the five populations was calculated as F_{ST} values using allele frequencies estimated in ANGSD. An unfolded site frequency spectrum (SFS) was estimated (-dosaf 1, -fold 0) for each population using realSFS as implemented in ANGSD (Nielsen, 2012). The estimated spectra were subsequently used to construct a bootstrapped (-bootstrap 100) pairwise SFS between each of the populations. During this latter stage the SFS was folded in realSFS (-fold 1) to account for the unknown ancestral state, and as a result the Magpie-Robin reference genome was supplied as both the reference (-ref) and the ancestral (-anc) genomes. The means of the pairwise SFS bootstrapped replicates were calculated and used as a prior to estimate global pairwise weighted F_{ST} values with realSFS (fst stats).

Global autosomal heterozygosity was calculated at the individual level using a folded SFS (-dosaf 1, -fold 1) for the estimation of homozygous and heterozygous sites across the genome, creating 20 bootstrapped replicates that were used to determine confidence intervals of the estimated SFS. In order to control for some of the bias introduced by coverage when heterozygosity was estimated at the individual level, a site coverage minimum of $4\times$ was used as a value below this threshold has been found to have an effect on diversity estimates (van der Valk *et al.* 2019).

Only individuals with an average genome-wide coverage of $5\times$ and above were included in the analysis of runs of homozygosity (ROHs) (136/141) as in Hooper *et al.* (2020). The largest 30 scaffolds for each of these individuals were selected for ROH analysis defined by a minimum length of 10 Mb. The longest scaffold was 57.83 Mb and the shortest was 10.48 Mb, with a total length of 0.73 Gb. A panel of SNPs for each island was generated as .ped files using ANGSD (-doPlink 2). These data were then fed into PLINK (v1.09beta5.4; Chang *et al.* 2015) to assess genome-wide ROHs (--homozyg). ROHs were called using the following thresholds: a maximum gap between consecutive SNPs set at 1000 kb (--homozyg–gap 1000), sliding windows set to 300 kb (--homozyg–window–kb 300), minimum number of SNPs for an eligible run set at 50 (--homozyg–density 50), a maximum of 30 missing sites allowed per window (--homozyg–window–missing 30) and five heterozygous calls allowed per window (--homozyg–window–het 5). From this output, F_{ROH} (autozygosity) was calculated as the cumulative length of an individual's ROH above 4 Mb as a proportion of the length of the used scaffolds (0.73 Gb). This method allows differentiation between ROHs that are 'identical by state' and 'identical by descent', as short ROHs (defined here as less than 4 Mb in the Magpie-Robins) are expected to exist with distant inbreeding and are therefore probably explained by 'identical by state'.

RESULTS

The five island populations exhibit clear population structure

Principal component analysis (PCA) revealed obvious clustering of the five islands, with greatest differentiation among the longest-standing translocations (Fig. 2; Cousin in 1994, Cousine in 1995 and Aride in 2002). The founder population of Frégate sits toward the centre of the clustering and on no axis do Frégate and Denis segregate. The first three principal components account for 20.9%, 17.5% and 10%, respectively, representing almost half (48.4%) of the total variation among populations. The axis of the first principal component shows that Cousin differs most from the other populations, as it has the highest degree of variation. PC2 (Fig. 2a) and PC3 (Fig. 2b) separate Aride and Cousine, respectively. Higher PCs do not show any biologically meaningful clustering (Fig. S2).

Mirroring this, the highest degrees of differentiation resulting from pairwise F_{ST} analyses were observed between Aride, Cousin and Cousine (unweighted F_{ST} values between 0.109 and 0.110, Fig. 2c). The F_{ST} values when comparing the population on the principal founder island (Frégate) to that on Denis, to the most recent translocation were, at 0.039, only approximately half of those obtained when compared with populations from the other three islands.

The results from NGSadmix analyses at $k = 4$ support the population structure observed in the
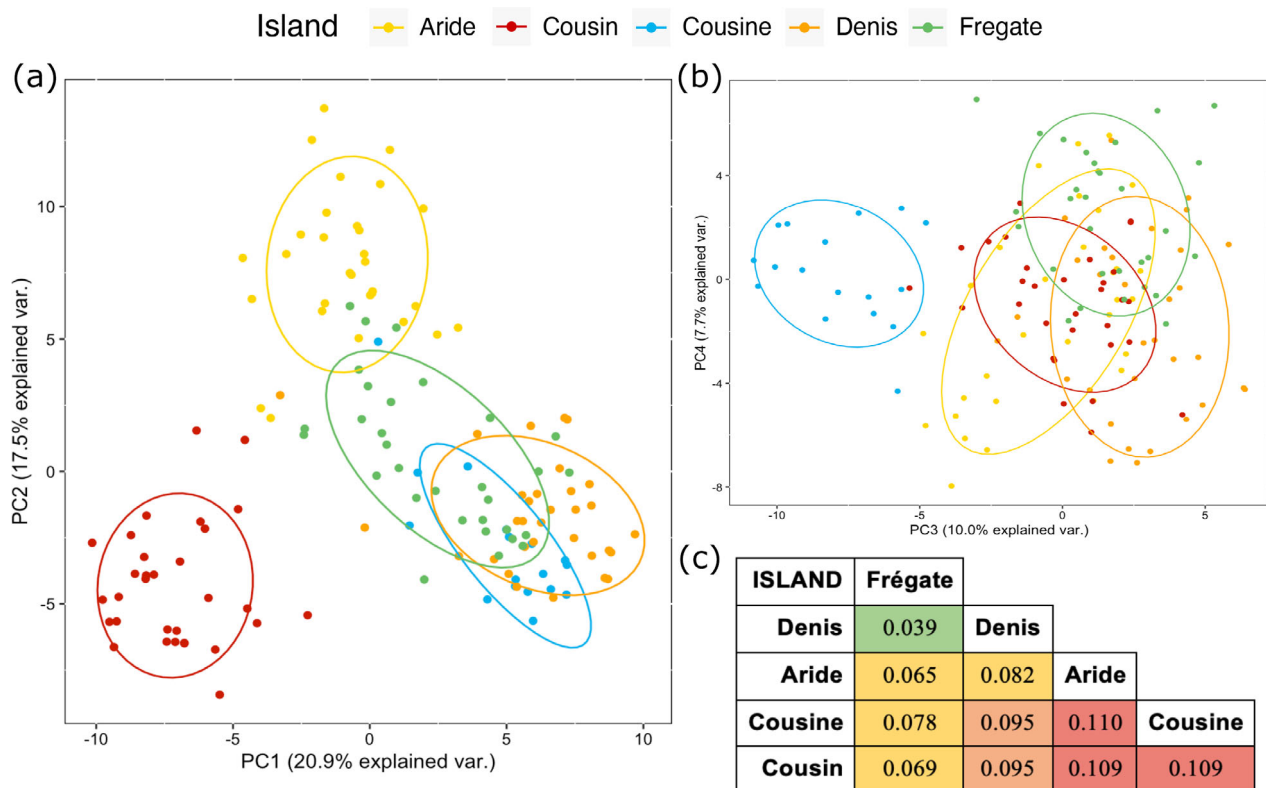
Figure 2. The five island populations exhibit clear population structure: (a) PC1 and PC2 from the principal component analysis, performed on the genotype likelihood from the full dataset (n = 141 individuals) using PCAngsd. (b) Same as (a) but showing PC3 and PC4. (c) Pairwise unweighted F_{ST} estimates computed with realSFS in ANGSD.

results of the PCA and F_{ST} analyses with Denis Island as the least genetically differentiated from the Frégate Island population. Structure through admixture is evident by a preponderance of one ancestral state in each population (depicted in Fig. 3 with 'island' colour) occurring systematically, with each increased k defining Cousin, Cousine and Aride at $k = 2$, $k = 3$ and $k = 4$, respectively. Interestingly, at $k = 5$, the differences within the Aride population show more variation than the other three translocated populations.

A species with very low genome-wide autosomal heterozygosity

The Seychelles Magpie-Robin genome-wide autosomal heterozygosity (global heterozygosity hereafter) was found to be exceptionally low, independent of population parameters. The average global heterozygosity (number of singletons/total number of sites) of each population was

around 0.00015 (Fig. 4a). This value is less than half the average value reported from similar analyses of 42 other IUCN red-listed avian species (Li *et al.* 2014). To illustrate a comparison of heterozygosity across threatened avian species Figure 4(b) uses nine of the published results in Li *et al.* (2014) together with the addition of our Magpie-Robin results.

Evidence of high inbreeding in the Seychelles Magpie-Robins

Only stretches of 4 Mb or longer were used to compute F_{ROH} (an estimate of inbreeding based on ROHs), as this length is an expected signal of historical inbreeding (Gibson *et al.* 2006, Simon-Sanchez *et al.* 2006, McQuillan *et al.* 2008). We observed not only that all populations exhibited ROHs of up to 8 Mb long, but also that every individual from Cousine and Denis Islands, and at least 80% of the individuals from the other islands,
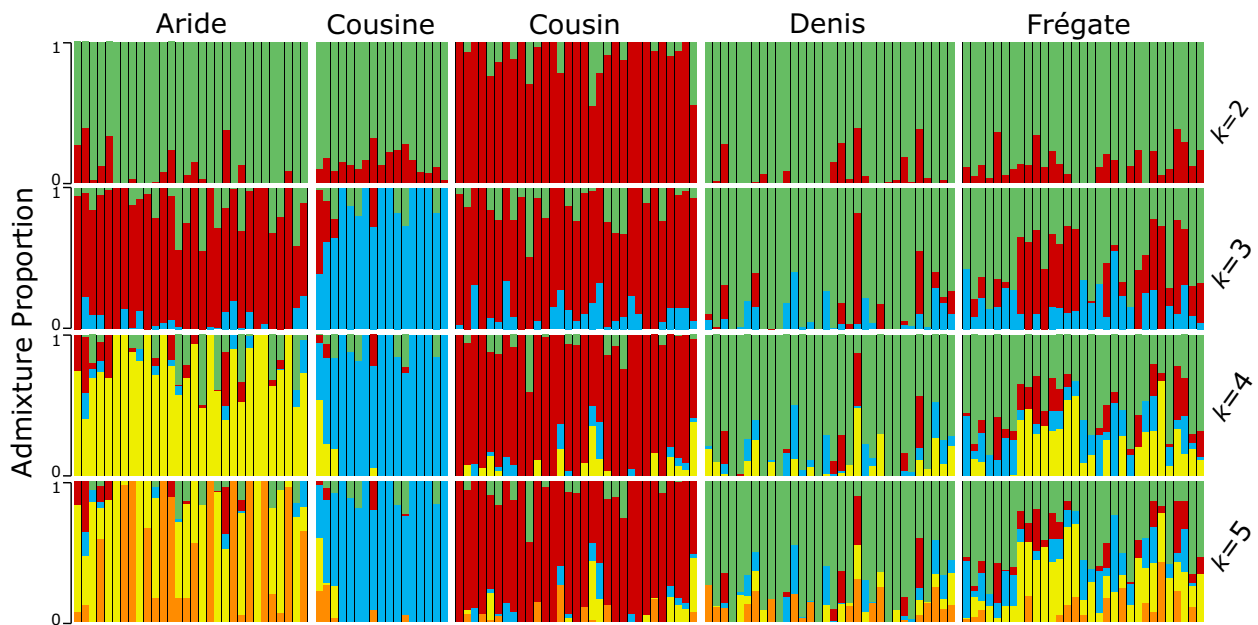
Figure 3. Population structure observed in the extant populations: NGSadmix results generated from the genotype likelihood data of all 141 individuals. Individuals are represented by columns, whereas rows depict the Admixture proportions based on the assumption of different numbers of ancestral populations. Displayed are results from $k = 2$ (top) to $k = 5$ (bottom).

had at least one ROH of 8–16 Mb (Fig. 5). Furthermore, at least half of all individuals on each island (Aride 59%, Cousin 63%, Cousine 70.6%, Denis 74.2%, Frégate 53.3%) exhibited at least one extremely long ROH (>16 Mb). With ROH shorter than 4 Mb excluded from 'identical by descent' interpretation, all populations were observed to have individuals with high F_{ROH}. Lastly, and consistent with the fact that these islands have a higher proportion of individuals exhibiting ROHs in the largest two categories, the Cousine and Denis populations also exhibited the highest median F_{ROH}, while Frégate Island shows the lowest F_{ROH} median.

DISCUSSION

This study provides the first comprehensive genomic assessment of the IUCN Red-Listed Endangered Seychelles Magpie-Robin. Our research aimed to answer fundamental questions about the current genetic state of the species using genome-wide SNP markers obtained from a sample set of 141 individuals representing the populations of the five islands within the Seychelles archipelago on which this species is extant. It is clear that the

recent history of the Seychelles Magpie-Robin has shaped the species' genome, with genomic signatures of population collapse and inbreeding mirroring to some degree the isolation of the five islands. The results are discussed here in relation to the genetic impacts of population history, past conservation translocations and the implications and applications of this research on the future conservation management of this, and other, endangered species.

Observed genetic impact of translocations

The Denis Island population has not yet been strongly affected by patterns of genetic drift, as it retains most genetic similarity to Frégate. This may reflect the larger size of the main donor population (92) at the time the individuals were taken, the number of individuals donated (16 from Frégate Island and four from Cousin Island, although only two of the latter survived the first year) and the relatively recent time since introduction (2008). Furthermore, during the second-year census only two of the birds moved from Cousin Island were found (S.M.A.R.T. pers.
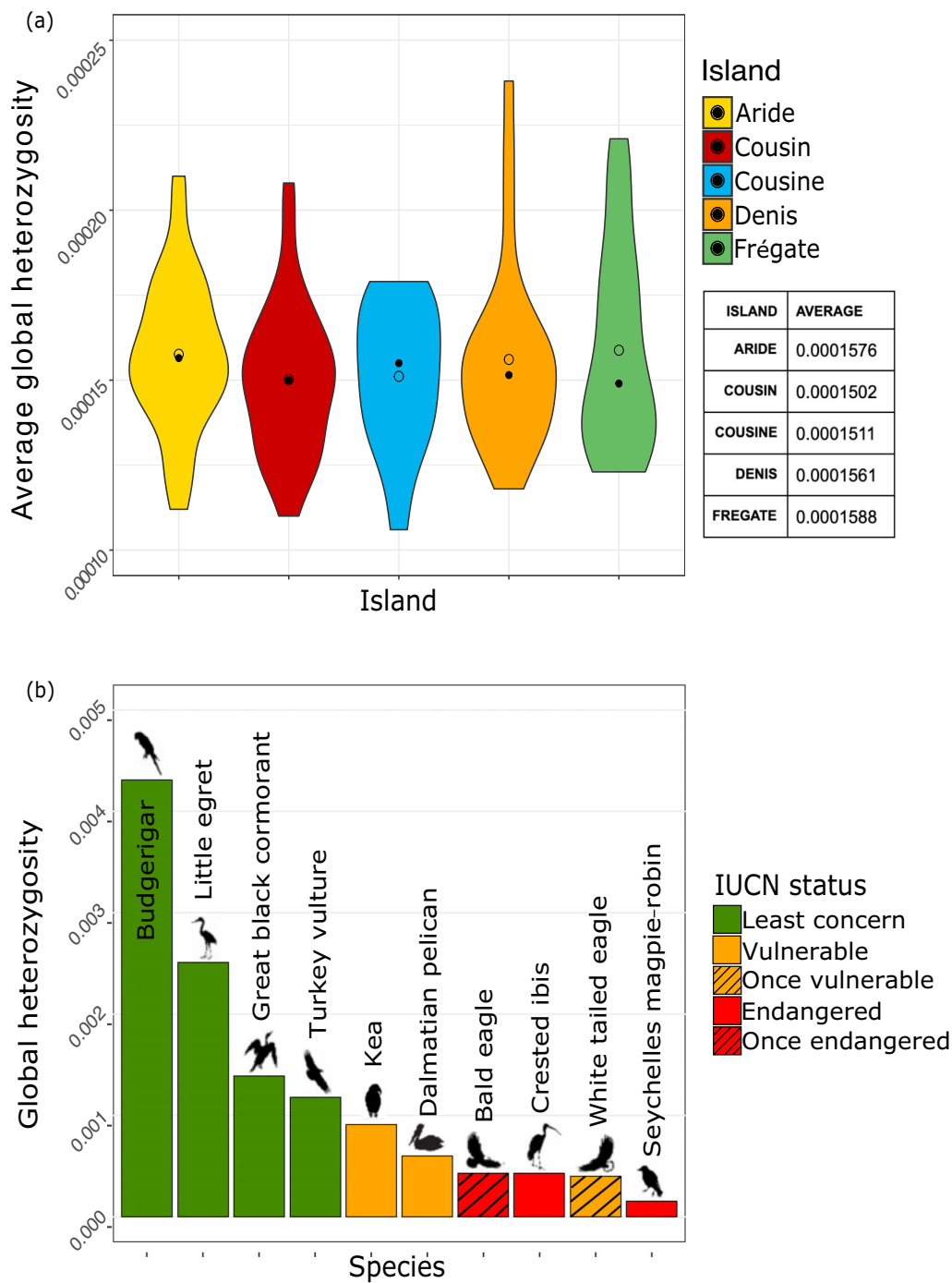
Figure 4. A species with very low genome-wide heterozygosity. (a) Global autosomal average heterozygosity estimates for each Seychelles Magpie-Robin sampled, grouped by island using violin, across all five Magpie-Robin island populations. The average is calculated from bootstrapped estimates for each Seychelles Magpie-Robin sample, giving an overview of island-level heterozygosity (open circle represents calculated mean, filled circle is calculated median). Inset shows the average heterozygosity estimate value for each island population. (b) Genome-wide autosomal heterozygosity estimates for 10 different IUCN Red-Listed avian species* – nine adapted from the results of Li *et al.* (2014) and the Seychelles Magpie-Robin estimates gained from this research – are incorporated for comparison. *Scientific name of included species reading left to right: *Melopsittacus undulates*, *Egretta garzetta*, *Phalacrocorax carbo*, *Cathartes aura*, *Nestor notabilis*, *Pelecanus crispus*, *Haliaeetus leucocephalus*, *Nipponia nippon*, *Haliaeetus albicilla*, *Copsychus sechellarum*.
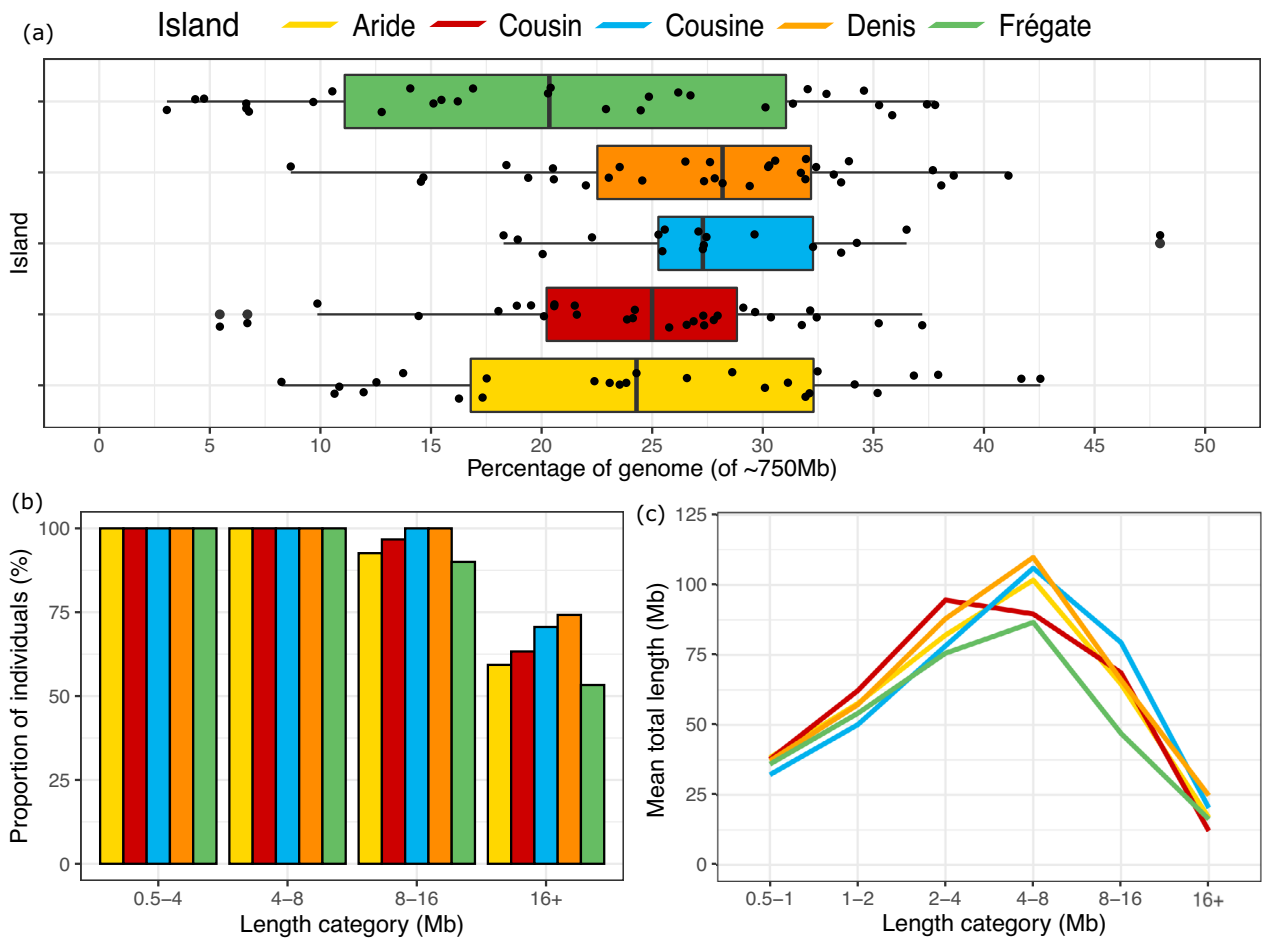
Figure 5. Evidence of high inbreeding in the Seychelles Magpie-Robin, found through analysis of runs of homozygosity (ROHs). (a) Island F_{ROH} (autozygosity) where only ROHs of 4 Mb long or above were included as a factor for 'identical by descent'. (b) Proportions of individuals of each island that possess ROHs of each size category. (c) Mean total length of ROHs by island for six length categories.

comm.), so the genetic contribution of birds from Cousin Island to the next generation appears to be limited.

A second striking observation is the considerably different levels of differentiation observed when comparing the Cousin and Cousine populations to their Frégate population source. We find this observation striking because it was recently suggested that the three sources are likely to be genetically one population (Burt *et al.* 2016), given that: (1) the translocation of individuals happened when Frégate was at its smallest population size (23 individuals), (2) the translocations happened within a very tight time frame (within 2 years) and (3) the close proximity of the three islands suggests that they are most prone to migrations. Nevertheless, it is clear from our results that the Cousin and

Cousine populations were probably affected differently by genetic drift. In this regard, we note that Cousin Island, which exhibits the strongest signal of divergence, experienced a period of severe weather between 2007 and 2008 that resulted in the loss of many birds (S.M.A.R.T. pers. comms.), and furthermore in 2008 four individuals were donated to the Denis Island translocation. Together these reduced the population by almost half (46 to 27 birds), rendering it much more susceptible to drift than the population of Cousine.

We thirdly observed that the genetic differentiation (F_{ST} analyses) observed between the Aride, Cousin and Cousine populations is probably highest because they are the longest established introduced populations. Furthermore, Cousin and Cousine received fewer birds donated from Frégate

at a smaller population size. Hence, the genetic variation between these populations appears to be a product of the population structure (PCA and admixture analyses) resulting from recent translocations. In a recent genetic study involving the Crested Ibis *Nipponia nippon*, a species with a similar history to the Magpie-Robins, an F_{ST} value of 0.134 was considered to be relatively high given the short time (20 years) since new populations were established (Li *et al*. 2014), which yields similar implications for the results of the Magpie-Robin (highest F_{ST} value of 0.110). The severity of genetic drift evident already in the Magpie-Robin populations despite the short time since translocations confirms the well-established idea that the evolutionary effects of drift act more quickly on small populations, although rapid selection has been documented to act on small populations, particularly when faced with adverse conditions (Lamichhaney *et al*. 2016).

The group of Endangered and Vulnerable species used in the avian genomics study of Li *et al*. (2014) found an average heterozygosity estimate of 0.0018 (calculated as the proportion of sites used that were observed to be heterozygous), with the lowest levels found in the Crested Ibis, Bald Eagle *Haliaeetus leucocephalus* and White-tailed Eagle *Haliaeetus albicilla* (0.00043–0.00040). In the Magpie-Robin, the very low level of observed genome-wide heterozygosity (0.00015) appears even more extreme than that of other threatened avian species (Li *et al*. 2014). However, this is more likely to be a feature of past genetic bottlenecks (1960s and earlier) than of the more recent translocations, given that the Frégate population harbours a level of heterozygosity similar to all other populations. The genetic differentiation observed in three of the four islands is indicative of an artefact of the recent translocations. Although estimates for the Magpie-Robins were made at the individual level, the purpose of this was to gauge whether global heterozygosity differs among islands.

The availability of a *de novo* sequenced, relatively high-quality Magpie-Robin reference genome assembly (75% of the genome comprises scaffolds of 10 Mb and above in length) and the genome-wide SNP dataset for a representative number of individuals from each island has allowed for reliable preliminary ROH analysis for the Magpie-Robins. However, as a full chromosome-level genome assembly does not yet exist for the Seychelles Magpie-Robin, any deeper interpretation should be applied with some caution. ROH analysis revealed extensive tracts of homozygous SNPs, a signature of recent inbreeding. Although the overall degree of relatedness generally appears high in each population, the length of the ROH can be used to infer recentness of inbreeding events and long ROHs (up to 56 Mb in some individuals; almost the entire length of the longest scaffold used) indicates that within each Magpie-Robin population, close familial mating has happened in recent generations (McQuillan *et al*. 2008, Kirin *et al*. 2010, Moyse *et al*. 2016).

In 2014, the Aride population underwent a catastrophic crash, with undetermined cause, and the population has since been unable to recover to more than a dozen individuals. One aim of this research was to ascertain if there may have been an underlying genetic cause for this crash, and whether assisted gene flow to diversify the gene pool should be considered for population recovery. As the low level of heterozygosity, the long length of ROHs and the high F_{ROH} are consistent between islands, this preliminary genomic investigation does not show an apparent genetic cause as to why this crash occurred (perhaps indicating a lack of recruitment or social conflicts as discussed in Burt *et al*. (2016), or perhaps an environmental cause). However, we note that one recent study demonstrated that the length of an ROH can be associated with the likelihood of harbouring deleterious variants (Sams & Boyko, 2018). Thus, given that very long ROHs were found in all five Magpie-Robin populations, future investigation into what genes are associated with the regions of long ROHs may reveal evidence of suspected inbreeding depression (i.e. alleles in which the homozygous recessive state would be lethal) in any of the five extant populations, which may not only give insight into past population size fluctuations, but could also serve as an additional consideration in conservation development for this species.

Application of genomics to conservation management

Translocations from small populations and/or endangered species are increasing in frequency, yet remain a contentious issue in conservation (Berger-Tal *et al*. 2020). Obtaining a genetic understanding of both the donor and the donated populations is required for initial planning and effective

management strategies. However, these considerations are rarely undertaken or monitored, because of time constraints, availability of resources or limited understanding of the impact it can have (Armstrong & Seddon 2008, Jamieson 2009). Extensive research into the impact of inbreeding on small populations has determined that inbreeding is associated with an increased extinction risk through a reduction in reproductive success and survival (O'Grady *et al.* 2006, Frankham *et al.* 2010), and inbreeding has been shown to have had a negative impact on the survival of island populations of passerines (Keller 1998, Jamieson 2009). Hence, quantifying individual inbreeding levels is integral to investigation of the effects of inbreeding in order to inform active management of populations. Using genome-wide markers for the ROH analyses carried out in this study has allowed for accurate quantification, and an essential genetic understanding, of such an inbred species.

In small, saturated populations it can prove difficult to ameliorate or break up ROHs, as there is limited mate choice. If long ROHs are not shared between isolated populations, implementing an assisted gene flow programme (i.e. genetic rescue) may help to increase the genetic diversity required for small populations to persist (Ralls *et al.* 2017), and thus could provide long-term benefits to participating populations (Saremi *et al.* 2019). A meta-analysis of genetic rescue conservation actions (translocating individuals of the same species between already established populations, when these populations are isolated and little gene flow exists between them) found that mostly positive benefits arose from diversification of the gene pool, and such actions seemed to combat the long-term effects of reintroductions through single-event translocations (Frankham 2015). In the context of our study, the very limited gene flow observed between these five Magpie-Robin populations, and hence the reproductive isolation after translocation, suggests that the Seychelles Magpie-Robin may make a good candidate for genetic rescue. However, although it may be possible to attempt such genetic diversification, it is important to note the potential risks associated with this approach, ranging from the transmission of unidentified diseases to the potential death of the donated individuals from either stress or failure to settle into the new environments.

In at least some of the previous Magpie-Robin translocations, the individuals were chosen for translocation using observational monitoring data to avoid moving closely related individuals. As our genetic data show that the populations today have a high level of background relatedness, it highlights an emphasis on the importance of molecular application to translocation donor selection. The new understanding of population-level genetics using the molecular insight provided by our study is expected to improve the success of future translocations by means of selectively maximizing genetic diversity for the Seychelles Magpie-Robin. Molecular understanding of inbreeding gained from this project can now be used as a genetic consideration for the proposed future conservation translocation of Seychelles Magpie-Robin in an attempt to establish a new, sixth, population on North Island, Seychelles (Millett *et al.* 2019).

Using either prospective or retrospective genetic monitoring, conservation genetics has already been partly attributed to the success of two other avian species in Seychelles (Bristol *et al.* 2013, Wright *et al.* 2014). Eleven of the 13 endemic avian species in Seychelles currently face some level of vulnerability (IUCN 2021), and using genetic resources to shape conservation action/policy should be seen as a natural addition to many recovery plans going forward, importantly, alongside maintaining direct actions of habitat restoration, removal of invasive species, protective legislation and public education.

In 2005, the Seychelles Magpie-Robin was downgraded from Critically Endangered to Endangered as the census population exceeded a threshold of 50 mature individuals (Birdlife International 2019). However, this was done with no prior genetic understanding of the species. Environmental stochasticity and the vulnerability of oceanic islands to such events, coupled with the suspected lack of genetic ability that the Magpie-Robin may have to adapt to these changes, could indicate a higher extinction risk than the species is currently categorized for. Although we would usually celebrate a reclassification considering a species to be less endangered, funding and research efforts were seen to plateau in conjunction with this change in conservation status. With a species with such small and variable population sizes, a lack of funds and resources to continue efforts to help save the Seychelles Magpie-Robin could prove fatal for the species, as with six other avian species that have gone extinct from this archipelago. Based on the example of our study, we advocate that if a species

is more vulnerable than currently defined, because of factors such as genetics, this needs to be duly considered in its IUCN conservation status.

AUTHOR CONTRIBUTION

Emily L. Cavill: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (equal). **Shyam Gopalakrishnan:** Formal analysis (supporting); Methodology (supporting); Supervision (supporting); Visualization (supporting); Writing-review & editing (supporting). **Lara C. Puetz:** Methodology (supporting); Supervision (supporting); Writing-review & editing (supporting). **Ângela M. Ribeiro**: Methodology (supporting). **Sarah S. T. Mak:** Methodology (supporting); Supervision (supporting); Writing-review & editing (supporting). **Rute R. da Fonseca:** Methodology (supporting); Writing-review & editing (supporting). **George Pacheco:** Methodology (supporting); Writing-review & editing (supporting). **Bronwyn Dunlop:** Resources (equal); Writing-review & editing (supporting). **Wilna Accouche:** Resources (equal); Writing-review & editing (supporting). **Nirmal Shah:** Resources (equal); Writing-review & editing (supporting). **Anna Zora:** Resources (equal); Writing-review & editing (supporting). **Licia Calabrese:** Resources (equal); Writing-review & editing (supporting). **Martin Genner:** Supervision (supporting); Writing-review & editing (supporting). **Gareth Jones:** Supervision (supporting); Writing-review & editing (supporting). **Chunxue Guo:** Resources (equal); Writing-review & editing (supporting). **Guojie Zhang:** Resources (equal); Writing-review & editing (supporting). **M. Thomas P. Gilbert:** Conceptualization (equal); Funding acquisition (lead); Resources (lead); Supervision (lead); Writing-original draft (supporting); Writing-review & editing (supporting).

COMPETING INTERESTS

None.

Data Availability Statement

All raw sequence read data have been deposited into the Sequencing Read Archive (SRA: https://www.ncbi.nlm.nih.gov/sra) and are accessible through project number PRJNA722144.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Table of summary statistics for sequencing data for each island population: the island mean is shown with standard deviation of the mean presented in brackets

Figure S1. Seychelles Magpie-Robin population trend estimates from 1973 to 2019. Data points are maximum count estimates of each year. Data compiled from Watson *et al.* (1992) and Burt *et al.* (2016), and provided by S.M.A.R.T. (unpublished bi-annual report 2019). (a) Total population count for all islands with coloured asterisk to denote date of the start of the most recent translocations to Cousin, Cousine, Aride and Denis Islands in 1995, 1995, 2002 and 2008 respectively. (b) Island population counts. Aride data include all translocation attempts and monitoring counts from 1978.

Figure S2. Higher PCs = no biologically meaningful clustering: Scree plot and PCA 5–10. (a)

Scree plot showing percentage of expected variances for each principal component. (b) PC5&6, (c) PC7&8, (d) PC9&10

Figure S3. Photograph of an adult Seychelles Magpie-Robin (left) feeding young offspring (right) one of their favourite snacks: a cockroach. Juveniles can be, quite literally, spotted by the brown flecks in the white bar on the wing, the duller black feathers which lack the characteristic blue sheen found on adults, and by the yellow gape around the beak. The red plastic ring visible on the right leg of the adult is the 'Cousin Island' identifier. The metal ring above it is embossed with a unique number that can be used to track birds ringed in Africa, supplied and administered by SAFring, the South African Bird Ringing Unit, FitzPatrick Institute of African Ornithology (http://safring.birdmap.africa). The left leg holds rings of a unique colour combination that make individuals easier to identify in the field, when carrying out behavioural monitoring. Photograph taken, and permission to use given, by Chris Tagg.