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RESEARCH

Genetic diversity and inbreeding in an endangered island-dwelling parrot population following repeated population bottlenecks

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Abstract

Genetic diversity and population structure can have important implications for the management of threatened species. This is particularly true for small, isolated populations that have experienced significant declines or population bottlenecks. The Norfolk Island green parrot *Cyanoramphus cookii* is an endangered species at risk of inbreeding and loss of genetic diversity due to its restricted range and the population bottlenecks experienced in recent decades. To assess the severity of inbreeding and loss of genetic diversity in the population we analyzed single nucleotide polymorphisms (SNPs) for 157 unique genetic samples collected from nestlings and randomly captured adult birds between 2015 and 2022. We also assessed the population for genetic structure, calculated sex ratios, and looked for evidence of past population bottlenecks. Our analysis revealed that 17.83% of individuals sampled were highly inbred ($F > 0.125$), although expected heterozygosity (H_E) did not significantly differ from observed heterozygosity (H_O) and the average inbreeding coefficient was low. The estimated effective population size (N_e) was 43.8 and we found no evidence of genetic structure. Demographic simulations provided support for scenarios including multiple population bottlenecks, when compared to those with a single population bottleneck or no past bottlenecks. We discuss the implications of our findings for the future management of the species including any potential attempt to establish an insurance population via translocation. Our study highlights the importance of considering population genetics when determining appropriate management actions for threatened species and the need to assess non-model species on an individual basis.

Keywords Birds · Conservation · Conservation ecology · Ecology · Genetic variation · Wildlife management

Introduction

A reduction in genetic diversity can be expected when populations become fragmented or experience a large decrease in population size (Frankham 1996; Montgomery et al. 2000; Willoughby et al. 2015). Due to a reduction in the number of potential mates, inbreeding becomes more likely, which can have a negative impact on fitness (inbreeding depression) (Liberg et al. 2005; Kennedy et al. 2014; White et al. 2015; Hedrick and Garcia-Dorado 2016; Vega-Trejo et al. 2022). Similar effects are experienced during population bottlenecks, in which population size is drastically reduced for a period of time before recovery (Nei et al. 1975). The consequences of a bottleneck on genetic diversity and inbreeding will depend on the minimum population size reached and how quickly it recovers (Frankham et al. 1999; Bellinger et al. 2003; Marr et al. 2006; von Seth et al. 2022). Bottlenecks can also impact a population after demographic recovery.

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For example, Briskie and Mackintosh (2004) found that among 22 species of New Zealand birds, those with recent population bottlenecks below 150 individuals had a significant increase in hatching failure.

In some cases, the consequences of inbreeding can be reduced or even reversed through genetic management (Hedrick and Garcia-Dorado 2016; Frankham et al. 2017; Ralls et al. 2020). In instances where translocation or reintroduction of a species is required, genetic studies can provide crucial information to ensure a viable population is established and maintained (Ottewell et al. 2014; Brockett et al. 2022). This information can also help to identify instances where genetic intervention (for example the introduction of individuals from other populations) may be necessary (Hogg et al. 2006; Whiteley et al. 2015), and ensure adaptive potential to contemporary threats such as climate change is maintained (Hoffmann and Sgrò 2011; Weeks et al. 2011). Therefore, an understanding of the amount and geographical distribution of genetic diversity is important when managing threatened species.

Islands have a disproportionately large number of extant threatened species and extinctions (Fernández-Palacios et al. 2021). Restricted ranges and long-term isolation exacerbate the impact of threatening processes such as introduced species, habitat loss and climate change (Leclerc et al. 2018; Fernández-Palacios et al. 2021). Furthermore, island endemics often have lower genetic diversity than their mainland counterparts due to small founding populations and lower overall population sizes, increasing their vulnerability to anthropogenic change and catastrophic events (Frankham 1998; Fernández-Palacios et al. 2021; Leroy et al. 2021). Thus, an understanding of the population genetics of threatened island endemics is particularly important for their conservation.

The Endangered Norfolk Island green parrot *Cyanoramphus cookii* (hereafter 'green parrot') is endemic to Norfolk Island, a small, island territory in the South Pacific Ocean (Department of Climate Change, Energy, the Environment and Water 2023). Habitat loss, the introduction of predators and competitors, and direct persecution have had a drastic impact on the species since European settlement, resulting in population bottlenecks reportedly as low as 17 individuals (Hermes et al. 1986; Hicks and Preece 1991; Hill 2002; Ortiz-Catedral et al. 2018). The exact nature and severity of population fluctuations since European settlement is not known; however evidence of drastic population contraction is noted as early as 1908 (Hull 1910) and the population appears to have remained relatively small for the rest of the century (Hicks and Preece 1991; Hill 2002). By 2009 the population had recovered to an estimated 240 birds (Dutson 2013), before decreasing to 46–92 birds in 2013 (Ortiz-Catedral 2013). While the population appears to have rapidly increased in size since this most recent bottleneck (Ortiz-Catedral 2013; Ortiz-Catedral et al. 2018; Skirrow 2018), the impact of past fluctuations in population size on the species' genetic diversity is unknown. Furthermore, it remains unknown whether the population operates as a single genetic unit or multiple separate units with limited mixing, and at what scale and extent this genetic structure may present itself. Although the small range of the species implies that spatial structure is unlikely to exist, previous studies have found evidence of fine-scale genetic structure occurring in avian populations across small areas (Garroway et al. 2013) and testing this possibility is important for conservation. As a non-model species, understanding both the genetic diversity and structure of the green parrot population is important to future management of the species and could also provide further insight into the population dynamics and management of other isolated species. We conducted population-wide genetic analysis using DNA samples collected between 2015 and 2022. We aimed to answer four key research questions: (1) How genetically diverse is the contemporary Norfolk Island green parrot population?; (2) What is the current effective population size?; (3) Is there spatial genetic structure in the population and, if so, to what degree and at what scale? and, (4) Does the contemporary population show evidence of past population bottlenecks?

Methods

Study area and study species

Norfolk Island is a small external Australian territory (approximately 35km^2), located more than 700 km from the nearest landmass of New Caledonia (Director of National Parks 2024). The island's natural vegetation consists primarily of dense subtropical rainforest of which the largest remnant patch occurs in the Mount Pitt Section of the Norfolk Island National Park (460 ha, Director of National Parks 2024). This also represents the core range of the Norfolk Island green parrot (Director of National Parks 2024). The green parrot has likely been isolated on Norfolk Island since members of the *Cyanoramphus* genus dispersed from New Caledonia to New Zealand via Norfolk Island (Boon et al. 2001). While the species was apparently abundant upon the establishment of a European settlement in the late 1700s (Phipps 1981), by 1908 it was already restricted to the steep gullies of Mount Pitt (Hull 1910). Extensive monitoring of the species has taken place in recent decades (Hicks and Greenwood 1989; Hill 2002; Ortiz-Catedral et al. 2018), but the small population and densely vegetated habitat make the species difficult to study, and much about the population remains unknown. The population genetics and dispersal

patterns of the species are not well understood. Long-term monogamy is assumed for green parrots, as the typical breeding system for parrot species (Toft and Wright 2015). Overall, the dynamics of the single population occurring on Norfolk Island remain unclear. For this study we collected genetic samples from wild birds found on Norfolk Island from both inside and outside of the national park.

Genetic sample collection

Genetic samples were collected by Norfolk Island National Park staff and researchers in 2015 ($n=48$), 2019 ($n=10$), 2020 ($n=36$), 2021 ($n=26$) and 2022 ($n=68$) on Norfolk Island. 185 samples were collected from 73 locations within the Norfolk Island National Park and Botanic Garden, and three were collected on private property from a single location (Fig. 1). All samples were packaged separately upon collection and stored at -4 °C as soon as possible. All samples were stored dry (with silica bead desiccant, where possible), apart from a single blood sample from 2022 which was stored in 70% ethanol (Table S1). Samples from 2015, 2019 and 2020 were collected as part of ongoing national park management and monitoring activities at known nest sites. Samples from 2021 to 2022 were collected as part of both ongoing national park management and monitoring activities at known nest sites and research activities performed under Australian National University Animal Ethics Committee approval (A2020_13).

Research activities in 2021 and 2022 involved the non-intrusive, opportunistic collection of genetic material (feathers) from recent predation events ($n=4$), recent nesting sites ($n=5$) or birds found deceased ($n=1$). In addition, in both 2021 and 2022 we mist-netted for adult birds within the national park. This involved the targeted use of call broadcast to attract nearby birds into a mist-net, where a blood or feather sample was taken prior to release. We moved systematically around the national park while mist-netting, to obtain a sample that represented birds from across their core range. In total, samples were obtained from 59 birds caught at 41 locations across 14 days (Fig. 1). For further detail on data collection by year, sample type, sampling method, storage method and location refer to Table S1 and Fig. 1.

DNA extraction, genotyping and molecular sexing

We extracted DNA from the genetic samples using a Monarch® Genomic DNA Purification Kit (New England BioLabs, Victoria, Australia) following the manufacturer's protocols for tissue and blood. We included a reagent-only negative control in each batch of samples to check for contamination in the extraction process. Following extraction, DNA was stored at -20 °C in EB buffer (10mM Tris-Cl, pH = 8.5). DNA from 188 samples were sent for sequencing by Diversity Arrays Technology (DArT, Canberra, Australia) using the DArTSeq approach, which employs a

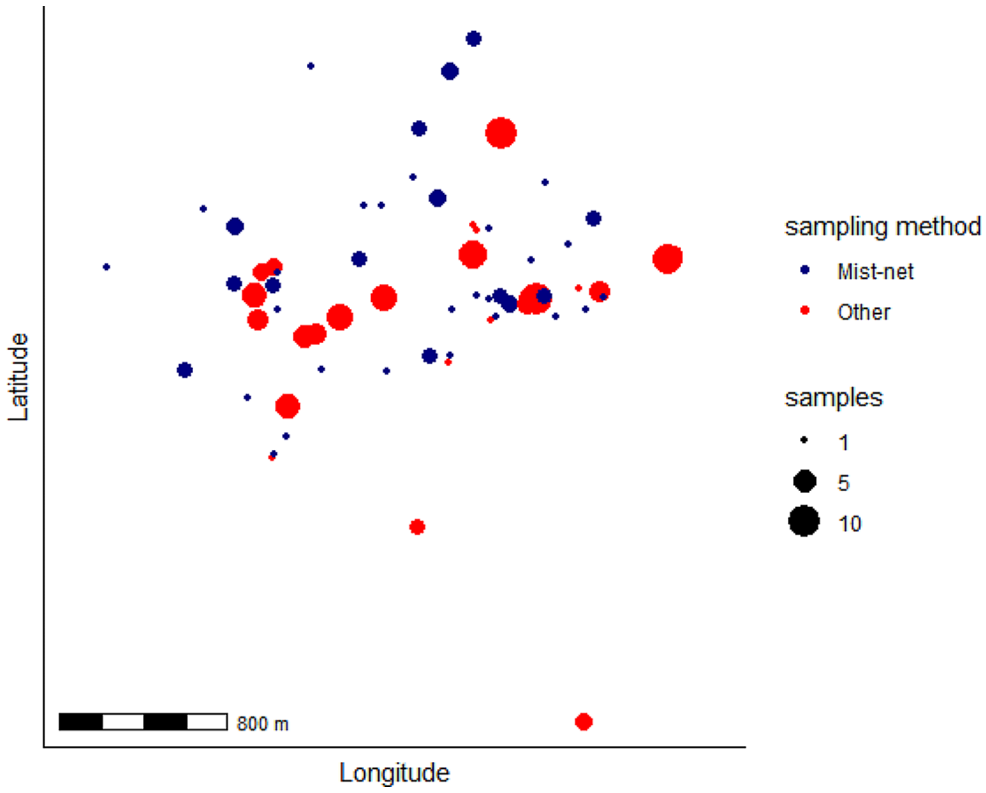


Fig. 1 Map of sample collection locations by number of samples and method of sample collection. Of the 188 samples processed, two samples from 2015 are not represented as exact collection coordinates were not collected

complexity reduction method to produce single nucleotide polymorphisms (SNPs; Kilian et al. 2012).

We performed molecular sexing on samples taken from nestlings ($n=67$) and adult birds captured in mist-nets in 2022 ($n=50$). We adapted the polymerase chain reaction (PCR) protocol described by Don et al. (1991) and Fridolfsson and Ellegren (1999). In each 12.5µL reaction, we used 0.25µL 2550F forward primer and 2718R reverse primer, 6.25µL OneTaq® DNA Polymerase (New England BioLabs, Victoria, Australia) and 1µL DNA. For initial testing we used an Eppendorf® Mastercycler Gradient machine, with annealing temperature ranging from 48 to 59 °C at 1 °C intervals, which identified 48 °C as the best temperature for the amplification of targeted genes on the Z and W chromosomes. For this process we included 3 of 41 nestling samples that were molecularly sexed in 2016 by The Equine Parentage & Animal Genetic Services Centre (Massey University, Palmerston North, New Zealand) as positive controls. We ran all PCRs on an Eppendorf® Mastercycler machine as described above and visualized them on a 1.5% agarose gel with sybr stain (Invitrogen, NSW, Australia), run for 30 min at 100 V. Males were identified as possessing a single product while females, as the heterogametic sex, amplify two products. To test whether sex ratios differed from expected proportions we used the 95% confidence intervals produced by the function *prop.test* from the stats package in R v4.2.1 (R Core Team 2023).

Data filtering

Individual SNP genotypes were called from sequence data by DArT using their proprietary bioinformatics pipeline. The data returned by DArT comprised 186 samples and 5,227 binary SNPs. We further filtered the dataset using the dartR package v2.0.4 (Gruber et al. 2018). Firstly, we filtered the SNP data to ensure quality by restricting read depth to between 5 and 31 and removing all sex specific loci. We then set the locus call rate threshold to 0.90 and the reproducibility threshold to 0.97 in order to keep only the best quality samples while maximizing the available sample size. In addition, we removed monomorphic loci, as well as secondary loci (in which there were 2 loci on the same tag), keeping the 'best' locus. We also removed loci with a hamming distance greater than 0.2. We set the minor allele frequency threshold to 0.01 following the formula presented in Lott et al. (2020). We did not perform Hardy-Weinberg filtering based on Pearman et al. (2022). We then applied an individual call rate threshold of 0.97 and removed any newly created monomorphic loci (see Code S1). This produced a genlight object with 1,356 binary SNPs and 167 genotypes.

We then used the package sequoia v2.3.5 (Huisman 2017) to identify duplicate genotypes in the filtered dataset ($n=167$). The dataset already contained three known duplicate samples (identified through bands on recaptured individuals) and four suspected duplicate samples. We adjusted the error rate until all known duplicates were successfully identified in sequoia (error rate = 0.02). In addition to the four suspected duplicate samples, we identified three other duplicates (Table S2, S3). When all else was equal we retained the most recent sample for further analysis. Once these 10 duplicates were removed from the genlight object, we repeated the filtering process, producing a dataset with 1,350 binary SNPs and 157 genotypes (referred to as Dataset A). Due to the nature of our sampling at known nest sites, the artificially elevated presence of closely related individuals (siblings and parent/offspring) could bias analyses of genetic structuring. As a result, we identified closely related individuals for removal. To do so, we constructed a full pedigree using sequoia (Stojanovic et al. 2022, Code S1). We used the resulting pedigree to create a second filtered dataset in which all individuals that made up a parent-offspring, full-sibling, half-sibling or grandparent-grandchild relationship, or were part of the same brood, were excluded. We retained the most recently obtained sample from the most recent generation when all else was equal, resulting in a dataset of 1,348 binary SNPs and 72 genotypes after the filtering steps were repeated (referred to as Dataset B). We used this dataset for the majority of our analysis, as we considered it the largest sample that was unbiased by the sampling methods employed as part of ongoing management of known nest sites in the national park.

Population diversity and effective population size

We explored population diversity using the dartR package. Our analysis primarily focused on expected heterozygosity (H_E) vs. observed heterozygosity (H_O), Wright's inbreeding coefficient (F_{IS}) (Wright 1949) and relatedness using genomic relatedness identity-by-descent (r). We estimated effective population size (N_e) using the linkage disequilibrium method from the NeEstimator v2 program (Do et al. 2014). We used default settings, producing N_e estimates and 95% confidence intervals. We calculated measures for both datasets. In addition, we estimated individual inbreeding coefficients using identity-by-descent (referred to here as F_{IBD}) in the package SNPRelate v1.30.1 (Zheng et al. 2012) for all unique samples that passed the first round of filtering ($n=157$).

Population structure

We explored the possibility of distinct clusters occurring within Dataset B ($n = 72$) using Pearson Principal Component Analysis (PCA) in the dartR package. We then used the function *find.clusters* from the package adegenet v2.1.7 (Jombart 2008), keeping sufficient principal components to explain 80% of variation and setting the maximum number of clusters to 10, in order to identify the most likely number genetic clusters within the sample.

We also tested for population structure in Dataset B using the program STRUCTURE v2.3.4 (Pritchard et al. 2000), using 5 iterations with the number of subpopulations (K) set between 1 and 5, a burn-in period of 5,000 and 10,000 repetitions. This was performed for scenarios under which allele frequencies were correlated among populations and independent among populations. We selected the best value of K for each scenario using the method of Evanno et al. (2005) and visualized this using the program CLUMPAK (Kopelman et al. 2015). As an additional test for spatial structure we used the package PopGenReport v3.0.7 (Adamack and Gruber 2014; Gruber and Adamack 2015), plotting genetic dissimilarity against geographical distance as well as spatial autocorrelation, both following the methods of Smouse and Peakall (1999).

Population bottleneck simulations

We generated a folded site frequency spectrum (SFS), reflecting minor allele frequency, to examine the likelihood of past population bottlenecks. These analyses require no missing data, thus we applied more stringent filtering to generate the SFS, which resulted in 1,603 SNPs for 122 individuals (see Code S1). We adapted the method of Afonso et al. (2023), using fastsimcoal2.7 (Excoffier et al. 2021) to test four population demographic models, which reflect simplified past population trajectories of the green parrot. These simulations reflected: (1) No population fluctuations (null model); (2) A single, 10-generation bottleneck reflecting dispersal of *Cyanoramphus* species to Norfolk Island approximately 450,000–600,000 years ago (Boon et al. 2001); (3) A single, 10-generation bottleneck since the arrival of Europeans on Norfolk Island (Hill 2002); and (4) Two bottlenecks, combining both scenario 2 and scenario 3. Bottlenecks comprised 10 to 100 individuals. Maximum likelihood values for each scenario were determined using 100 individual runs with 50 optimization cycles and 100,000 iterations (see Code S2). To determine the number of generations used to approximate the timing of historical bottlenecks, we considered generation time to be 6.1 years based on the estimates for the most closely related species, the red-crowned parakeet (*Cyanoramphus novaezelandiae*; Bird et al. 2020). To determine the most likely of the tested scenarios we calculated AIC values for each scenario using the Bash script *calculateAIC.sh*. The range of parameters tested are presented in Table S4.

Results

Molecular sexing

We identified two duplicates among the 67 nestling samples and one sample could not be sexed, leaving 64 unique nestling samples with a molecular sex assigned, in addition to the 41 nestling samples collected in 2015 and sexed in 2016 by The Equine Parentage & Animal Genetic Services Centre (Massey University, Palmerston North, New Zealand). Of these, 54 (51.43%) were male and 51 (48.57%) were female, representing an even sex ratio (male 95% confidence range = 0.42–0.61, $p = 0.85$). Only one of the 50 birds captured in mist-nets in 2022 was female, representing a highly significant male sex bias (98%, male 95% confidence range = 0.88–1.00, $p = < 0.001$).

Recaptures

Through banding information and matched genotypes in sequoia, we identified 10 instances of recapture (Table S2, Table S3). An additional six samples were the result of accidental resampling of individuals. Eight birds were recaptured in mist-nets after being sampled as nestlings. Of these, one bird was recaptured in both 2021 and 2022. All recaptures were male, with an average age of 5.5 years (range = 1.6–7.7 years, $n = 9$, Table S3). The average distance between birthplace and recapture location was 568 m (range = 88–1,144 m, $n = 9$, Table S3).

Diversity and effective population size

The observed heterozygosity (H_O) values of both datasets did not differ significantly from expected heterozygosity (H_E) values (Table 1). Nor did H_O or H_E differ significantly between datasets. Average relatedness (r) was slightly lower for Dataset B (0.033, range: 0–0.648) than Dataset A (0.044, range: 0–0.762). Both datasets had F_{IS} scores close to zero (Dataset A = 0.057, Dataset B = 0.052), suggesting little evidence of inbreeding (or heterozygote deficit). However, 20 of 157 individuals had a F_{IBD} between 0.125 and 0.25, suggesting that 12.74% of birds are highly inbred (according to inbreeding thresholds used by Laws and Jamieson 2011; Flanagan et al. 2021). Eight individuals (5.1%) had a F_{IBD} exceeding 0.25, suggestive of very high levels of inbreeding. The mean F_{IBD} was 0.068 (range = 0–0.4).

Table 1 Genetic diversity measures for both Norfolk Island green parrot datasets: number of samples (n), expected heterozygosity (H_E) and observed heterozygosity (H_O) ± standard deviation (SD), inbreeding coefficient (F_{IS}) and effective population size (N_e) and 95% confidence intervals

Dataset	n	$H_E \pm$ SD	$H_O \pm$ SD	F_{IS}	N_e (95% CI)
All birds (A)	157	0.322 ± 0.145	0.305 ± 0.142	0.057	25 (22–28.2)
All unrelated birds (B)	72	0.325 ± 0.147	0.310 ± 0.146	0.052	43.8 (38.9–51.2)

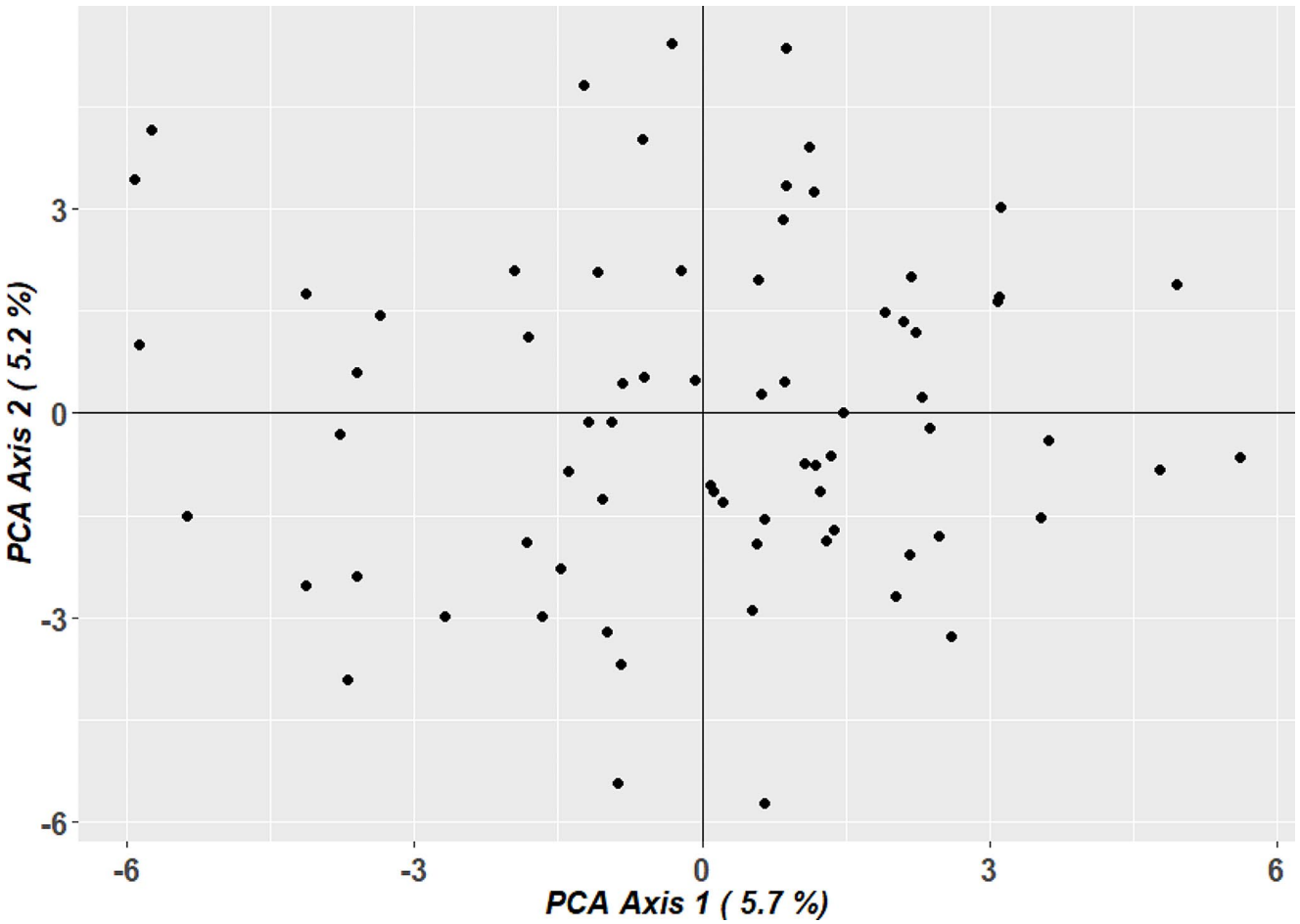


Fig. 2 Analysis of principal components for all unrelated Norfolk Island green parrots ($n = 72$). Numbers in parentheses denote the percentage of total variation explained by Principal Component Axes 1 and 2

Estimated effective population size was significantly lower for Dataset A (N_e = 25, 95% CI = 22–28.2, $n = 157$) than Dataset B (N_e = 43.8, 95% CI = 38.9–51.2, $n = 72$, Table 1).

Population structure

Principal component analysis showed no genetic clustering (Fig. 2), which was confirmed by a discriminant analysis of principal components that estimated the most likely number of subpopulations (K) to be 1 (Fig. S1). Outputs from the program STRUCTURE estimated K to be 2 ($\Delta K = 23.49$) when allele frequencies were correlated among populations, and 3 ($\Delta K = 96.72$) when allele frequencies were independent among populations. However, given ΔK is a relative measure it cannot be calculated for $K = 1$ (Janes et al. 2017), and further inspection indicated very few individuals were convincingly assigned to a population at $K = 2$ or $K = 3$ (Fig. S2). Spatial autocorrelation was not significant for Dataset B ($n = 72$, Fig. 3).

Population bottleneck simulations

Coalescent simulation testing provided greatest support for a scenario containing both recent and ancient bottlenecks (ΔAIC compared to a scenario with no bottlenecks (null) = -7,699.9), followed by a scenario containing only a recent bottleneck (ΔAIC = -6,530.7). The scenario containing only

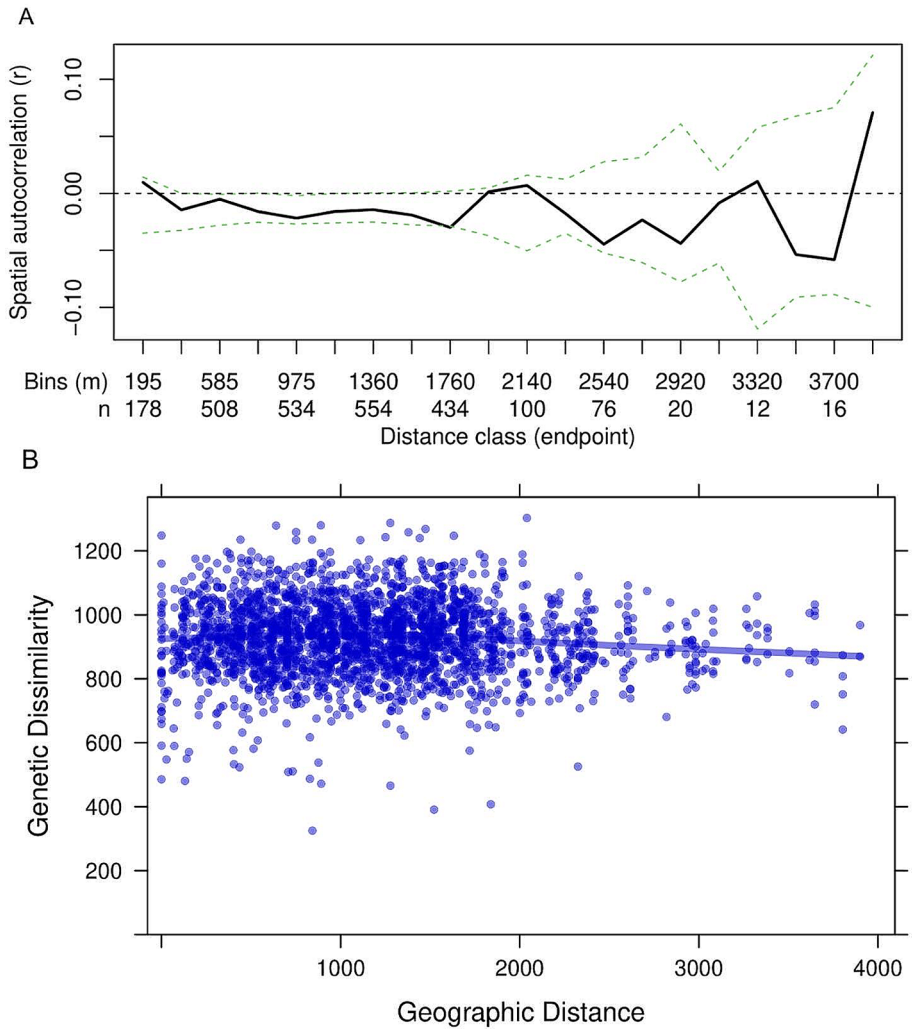


Fig. 3 **A** Spatial autocorrelation (r) prediction (solid black line) with 95% null hypothesis confidence intervals (dotted green lines) and **B** the relationship between genetic dissimilarity (from Smouse and Peakall (1999)) and geographic distance (m) for all unrelated green parrots (Dataset B, $n = 72$)

an historical bottleneck (ΔAIC = 23.87) was significantly worse than the null model (Table S5). The maximum likelihood parameters from the most likely scenario tested suggest a small founding population on Norfolk Island ~ 50,000 generations ago (~ 310,000 years ago), a population of ~ 10,000 birds prior to the arrival of Europeans, followed by another bottleneck ending one generation ago, with a current population of ~ 700 birds. However, the maximum log likelihood of the estimated values was not close to the maximum log likelihood of observed values, indicating a poor fit (Table S5).

Discussion

Understanding the extent of inbreeding and loss of genetic diversity is fundamental when managing small, isolated populations (Frankham 1998; Willoughby et al. 2015). This is particularly important for island endemics that have experienced past population bottlenecks or significant population contraction (Briskie and Mackintosh 2004; von Seth et al. 2022). Wright's inbreeding coefficient (F_{IS}) was near zero for both green parrot datasets, providing little evidence of inbreeding (or heterozygote deficit) (Frankham et al. 2002). This is supported by the overlapping standard deviation between expected and observed heterozygosity, regardless of the dataset used, and suggests that overall loss of genetic diversity in the population is limited. The average coefficient of relatedness (r) between birds in Dataset B was low (0.033). However, the maximum value remained high (0.648) despite the removal of sibling, offspring and grand-offspring relationships from Dataset A, indicating that the population is not inbred, but there is potential for some inbreeding within the population, likely due to recent bottlenecks. Similarly, we found that 17.83% of the birds sampled were highly inbred ($F_{IBD} \geq 0.125$), of which 5.1% were very highly inbred ($F_{IBD} \geq 0.25$) (according to inbreeding thresholds used by Laws and Jamieson 2011; Flanagan et al. 2021). The inbreeding coefficients found here (mean F_{IBD} = 0.068, F_{IS} = 0.057 (Dataset A) and F_{IS} = 0.052 (Dataset

B)), fall within the range of inbreeding coefficients (F) reported for other threatened parrot populations (Stojanovic et al. 2018, 2022) and isolated avian populations (Jamieson et al. 2007; Brekke et al. 2010; Laws and Jamieson 2011; Nietlisbach et al. 2017). Examples include Forbes' parakeet *Cyanoramphus forbesi* ($F = 0.15$, Chan et al. 2006), the kākāpō *Strigops habroptilus* ($F = 0.09$, Foster et al. 2021), the cape parrot *Poicephalus robustus* ($F = 0.039$, Coetzer et al. 2020), the Haast tokoeka *Apteryx australis* 'Haast' ($F = 0.102$, White et al. 2018) and the takahē *Porphyrio hochstetteri* ($F = 0.089$, Grueber and Jamieson 2008). While the F_{IBD} for some individuals in our dataset was very high (maximum $F_{IBD} = 0.4$), some severely bottlenecked species display significantly higher average inbreeding coefficients than the green parrot. For example, Chatham Island black robin *Petroica traversi* pairs were estimated to have an average inbreeding coefficient of 0.36 (Kennedy et al. 2014). It should be noted however that different sequencing and estimation methods used to calculate genetic relatedness often produce different results, making comparisons difficult (Hauser et al. 2022). Nonetheless, the number of highly inbred individuals in our sample is undesirable due the potential for inbreeding depression in future generations (Hedrick and Garcia-Dorado 2016; Vega-Trejo et al. 2022). Our results indicate that the small size of the population and the small distribution of the species may have had negative genetic effects.

Dataset A (containing all individuals) produced a mean N_e of 25 (95% CI; 22–28.2, $n = 157$), significantly lower than Dataset B (containing all unrelated individuals, $N_e = 43.8$, 95% CI; 38.9–51.2, $n = 72$), likely due to the large number of closely related birds included in Dataset A. Given the biased nature of this larger sample, caused by the repeated collection of genetic data from the same nest sites over time and therefore the sampling of many closely related individuals, we consider the estimate for Dataset B ($N_e = 43.8$) to be most representative of the contemporary green parrot population. Given the species' recent population bottleneck (Ortiz-Catedral 2013), small population size throughout the 20th century (Hull 1910; Hermes et al. 1986; Hill 2002) and the lower N_e values typically observed for island species (James et al. 2016; Leroy et al. 2021), an estimated N_e of 43.8 is larger than initially expected. This compares favourably with contemporary N_e estimates from other severely bottlenecked island populations with small contemporary populations, such as the takahē ($N_e = 12.8–44.6$, Grueber and Jamieson 2011) and the South Island saddleback *Philesturnus carunculatus* population on Motuara Island ($N_e = 12.7–34$, Knafler et al. 2017). Other avian populations endemic to islands exhibit considerably higher estimated N_e, reflecting their different histories and ecology (Nadachowska-Brzyska et al. 2021). For example, Cowles et al. (2021) estimated an N_e of 694.5 for the Solomons white-eye *Zosterops kulambangrae* and 796.1 for the Kolombangara white-eye *Zosterops murphyi*, both of which are endemic to islands of the Solomon Archipelago. However, both species have a large contemporary population and are common throughout their ranges (Cowles et al. 2021). Our N_e estimates are also relatively low when compared with other threatened parrots, such as the swift parrot *Lathamus discolor* ($N_e = 44–140$, Olah et al. 2021), superb parrot *Polytelis swainsonii* ($N_e = 62–75$, Stojanovic et al. 2022) and cape parrot ($N_e = 289.1–600$, Coetzer et al. 2020), which have also experienced significant population declines, but persist in greater numbers than the green parrot, across a larger contemporary range. Compared with an ideal population, our estimated N_e is very small, falling well below the 100 individuals suggested as the minimum N_e required to avoid inbreeding depression in the short term (Frankham et al. 2014).

We found no evidence of genetic structure in the green parrot population. While population structure has been found in birds across relatively small geographic areas (e.g. Woltmann et al. 2012; Garroway et al. 2013), the ease with which green parrots can move across the island makes extensive mixing possible. However, it remains possible that underlying structure was not able to be identified using our sample, given the high number of closely related birds in our larger sample and the inability to capture an adequate sample of adult females. A more robust and unbiased sample may allow for a more thorough inspection of fine-scale genetic structure.

Demographic simulations provided the most support for the occurrence of both an historical and recent population bottleneck in the green parrot population. However, our most likely model was a poor fit for the observed data, suggesting our simulations were not an accurate representation of the green parrot's past. This is unsurprising given the many unknown aspects of the species' history, including the original method and timing of dispersal to Norfolk Island (Boon et al. 2001) and the full nature and extent of population fluctuations since the arrival of Europeans on Norfolk Island (Hill 2002). In this analysis, we did not attempt to determine the exact past of the green parrot population, but rather aimed to examine the contemporary genetic samples for evidence of past population bottlenecking. Our results do suggest that multiple population bottlenecks are more likely than a single bottleneck, or no bottlenecks, which supports past observations and research (Hull 1910; Hicks and Preece 1991; Boon et al. 2001; Ortiz-Catedral 2013).

Molecular sexing using PCR techniques suggested an extreme male sex bias in birds captured in mist-nets (98% male, $n = 50$), alongside an even sex ratio at hatching (51.43% male, $n = 105$). Among birds facing increased

nesting-related mortality, a male sex bias is to be expected, given the additional risk assumed by incubating females (Donald 2007). However, the extreme sex-bias we observed is surprising. Even swift parrots, which face intense female-biased predation pressure, have a far more moderate adult sex ratio of 73–75% males (Heinsohn et al. 2019). Despite the likely increase in nest predation faced by incubating female green parrots (Hicks and Greenwood 1989; Hill 2002), the extreme nature of our observed sex ratio indicates that our results may instead be an artefact of our capture method. This phenomenon has been observed in other birds (Amrhein et al. 2012), particularly when using call broadcast to attract individuals (Lecoq and Catry 2003; Ndlovu 2018). The inability to capture female green parrots was also noted by Hicks and Greenwood (1989), when attempting to establish a captive breeding population. As our technique relied on the use of call broadcast, the best explanation for our results, at least in part, is that male birds were more likely to respond to call broadcast. One possible interpretation of our findings is that they support previous observations of skewed sex ratio, for example the 1:3 (female to male) sex ratio observed by Ortiz-Catedral (2013). On the other hand, they may instead cast doubt about observational studies of sex ratios in this species if males have higher detection likelihoods in general. This uncertainty can only be resolved by finding survey methods to accurately determine the adult sex ratio in the green parrot population.

Recaptures in our dataset provide new insight into lifespan and dispersal patterns of wild green parrots. Males were not captured far (88–1,144 m) from their natal location, conforming with the commonly observed lack of natal dispersal in male birds (Greenwood and Harvey 1982). However, our sample is very small, limiting interpretation, and no recaptured female birds were available for comparison. It should be noted that the limited extent of Norfolk Island's remaining native forest may discourage birds from moving as much as they would in an undisturbed landscape. In addition, Irwin et al. (2021) observed male-biased post-fledgling dispersal in the green parrot's closest relative, the red-crowned parakeet. Thus, further data are required to determine dispersal patterns in green parrots. One recaptured bird was first sampled as a nestling 7 years and 8 months prior, slightly exceeding the maximum longevity estimate of 7.3 years of age presented in Garnett et al. (2011), but less than the modelled estimate for the red-crowned parakeet of 9.53 years (Bird et al. 2020).

Our findings have several implications for the ongoing management of the Norfolk Island green parrot population. Past population bottlenecks have contributed to inbreeding levels that may cause inbreeding depression in the short term (Franklin 1980; Frankham et al. 2014). The species cannot afford to lose genetic variation and therefore efforts to maintain its diversity should be a focus in the coming years. In recent decades, alongside the ongoing control of invasive predators, a focus of management for the species has been the development and monitoring of predator-proofed nest sites (Hicks and Greenwood 1989; Ortiz-Catedral et al. 2018). However, only some of these nest sites are used by green parrots, and those that are used are often used repeatedly (Gautschi et al. 2022). Focusing too heavily on a small number of managed nest sites may exacerbate reproductive skews, and limit the diversity of genes passed to future generations (Stojanovic et al. 2022). For green parrots, this can be avoided by ensuring as many pairs as possible have access to suitable nest sites, through the maintenance of existing managed nest sites, and intensified control of introduced rats and cats to ensure pairs nesting in unprotected nests are also able to breed successfully. This can help to ensure that more parrots contribute genetically to future generations, and enable more rapid population growth overall. Since there are no other green parrot populations, genetic supplementation would not be possible without hybridisation as was done in desperate circumstances for the Norfolk boobook *Ninox novaeseelandiae undulata* in 1987 (Olsen 1996). To conserve the green parrot, translocation to a predator free island has been proposed as a possibility (Hicks and Greenwood 1989; Hill 2002; Ortiz-Catedral et al. 2018). To do so, sufficient diversity in the founding population would be required to set up a viable population (Jamieson 2011). Given the existing inbreeding we have observed, the potential impact of a translocation on both the original and translocated population should be considered (Morrison et al. 2020). Ongoing genetic monitoring would be required and possibly genetic supplementation of the new population over time (Ottewell et al. 2014). Furthermore, our study has highlighted the difficulty of capturing adult females, which have generally been included as part of successful translocations of related *Cyanoramphus* species (Collen et al. 2014). These findings would need to be considered in the event of a translocation attempt for the green parrot, alongside other common difficulties encountered during animal translocations (Berger-Tal et. 2020).

Overall, our findings highlight the need to consider population genetics when designing and implementing conservation management. By developing an understanding of genetic diversity and structure, researchers and practitioners can refine existing approaches and inform future management actions to promote the best possible outcomes for threatened species. This may be particularly important when attempting to conserve small, isolated populations facing ongoing threats. Furthermore, we show the importance of the individual assessment of population genetics for non-model species in order to understand their unique situation and tailor management actions accordingly.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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