IBIS
BOU international journal of avian science
Ibis (2022)
doi: 10.1111/ibi.13048

Short Communication

Structured phylogeography and restricted gene flow among populations of Fairy Tern (*Sternula nereis*) across Australasia: implications for the endangered New Zealand population

MARLEEN BALING*[1,2] & DIANNE H. BRUNTON[1,3]
[1]*School of Biological Sciences, The University of Auckland, Auckland, New Zealand*
[2]*School of Environmental and Animal Sciences, Unitec Institute of Technology, Auckland, New Zealand*
[3]*School of Natural and Computational Sciences, Massey University, Auckland, New Zealand*

The Fairy Tern *Sternula nereis* is an Australasian tern that breeds in Australia, New Caledonia and New Zealand, with the last having the smallest breeding population, listed as 'Threatened – Nationally Critical' by the New Zealand Department of Conservation. Here, we investigate the genetic relatedness and level of endemism (gene flow) of the New Zealand Fairy Tern *S. n. davisae* population compared with the larger breeding populations in Australia, *S. n. nereis*, and New Caledonia, *S. n. exsul*, using the NADH subunit 2 (ND2) region of the mitochondrial DNA. We found that the three main populations (n = 86) were genetically distinct, with a different fixed haplotype restricted to New Zealand (n = 15) and New Caledonia (n = 16), and that the estimated gene flow was low to zero, indicating no interbreeding between the populations. The current genetic evidence is consistent with observations of morphological and behavioural differences among the populations, and we suggest continued independent management of the population in New Zealand and further surveys and independent management of the New Caledonia population.

Keywords: Australia, conservation genetics, New Caledonia, seabird.

*Corresponding author.
Email: m.baling@gmail.com
Twitter: @drmarleyb

The Fairy Tern *Sternula nereis* is an Australasian species, ranging widely from Australia to New Caledonia and several other Pacific Islands (Tarburton 2001, Borsa *et al.* 2010, BirdLife International 2018) (Fig. 1a). There are three main breeding populations, distributed across three countries, each with a geographically restricted population/subspecies of Fairy Tern: *Sternula nereis nereis* (Australia), *Sternula nereis exsul* (New Caledonia) and *Sternula nereis davisae* (New Zealand) (Higgins & Davis 1996). The current subspecies status is based on morphological differences and geographical distances of the main breeding populations from each other (Hitchcock 1959, 1967, Hill *et al.* 1988, Higgins & Davis 1996).

The New Zealand Fairy Tern *S. n. davisae* has the smallest population size, with *c.* 29 individuals and *c.* 11 active breeding pairs (three breeding pairs in 1983, Ferreira *et al.* 2005). This population is endemic to New Zealand and is considered one of the rarest and most threatened birds in New Zealand (Parrish & Honnor 1997, Taylor *et al.* 2004, Hansen 2006). The current national conservation status is 'Threatened – Nationally Critical' (Robertson *et al.* 2017). However, the New Zealand subspecies of Fairly Tern has received little attention internationally despite its alarmingly low population size because the overall species status is listed as 'Vulnerable' in the IUCN Red List (BirdLife International 2018). This perceived lower conservation value is due to the general assumption that the New Zealand population is a recent colonist to the country and is thereby considered a peripheral population of the larger and more stable Australian population (Kinsky 1970, Falla *et al.* 1979, BirdLife International 2018).

Currently, it is unknown whether the breeding populations in Australia, New Zealand and New Caledonia interbreed. Some colour-ringed New Zealand Fairy Tern fledglings and adults that were recorded absent for several years appeared again at later dates at known breeding sites and successfully bred in New Zealand (Ferreira *et al.* 2005). It is possible that the terns are moving large distances outside of the commonly monitored areas, potentially migrating out of New Zealand. Such long-distance movement has been observed in New Caledonia Fairy Terns, which have appeared along the northern coast of Australia (Hill *et al.* 1988, Higgins & Davis 1996, Garnett & Crowley 2011). However, there has been no observation of a colour-ringed New Zealand Fairy Tern mixing with either the Australian or the New Caledonian breeding populations.

Previous genetic research (12S ribosomal RNA and cytochrome *b*) showed that New Zealand and Australian Fairy Terns had low levels of differentiation between the two populations (Coddington 1996, Chambers & Coddington 1998). Partial sequences of cytochrome *b* (257 bp) showed only one nucleotide substitution between New Zealand (n = 1) and Australian (n = 1)

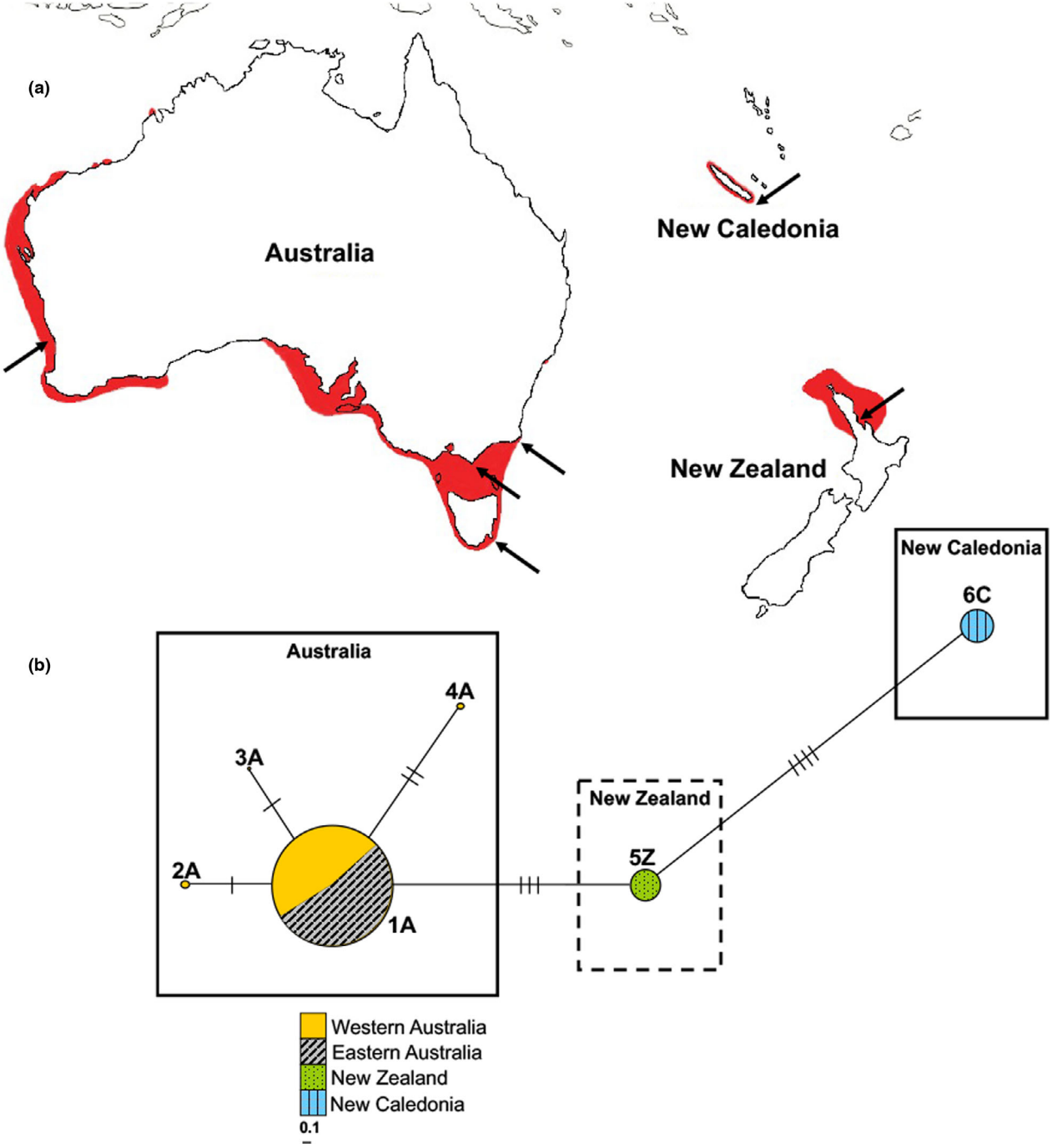

Figure 1. (a) Distribution of Fairy Terns (in red, modified from Birdlife International, 2018). Arrows indicate locations where tissue samples were collected during the 2003–2004 season. (b) Minimum spanning network of the six ND2 haplotypes of Fairy Tern found in three countries: Australia (subpopulations Western Australia $n = 31$, Eastern Australia $n = 24$), New Zealand ($n = 15$) and New Caledonia ($n = 16$). The sizes of the circles indicate the sample size for each population, with the proportions marked for each subpopulation for Australia. The lengths of the connecting lines between the circles are distances calculated according to pairwise differences. The number of bisecting lines on the connecting lines indicates the number of nucleotide substitutions separating haplotypes.

Fairy Terns (Coddington 1996). Later, a partial sequence of 12S rRNA (382 bp) was also obtained, but at that time the authors did not have sequences from closely related species to confirm that the sequence was Fairy Tern (Chambers & Coddington 1998). In summary, the sample sizes were small for these studies and did not include samples from the New Caledonia population, making it hard to determine the taxonomic status of the New Zealand Fairy Tern.

The objectives of our study were to assess the genetic variability of Fairy Terns within and among New Zealand, New Caledonia and Australia, and to estimate the level of migration per generation (female gene flow) among the populations. We expected that genetic variation would be higher in the larger mainland populations than in the smaller breeding island populations and that there would be some degree of genetic isolation of the New Zealand population.

METHODS

Study sites and fieldwork

We analysed Fairy Tern populations from four locations: New Zealand, New Caledonia, Western Australia and southeast Australia. The Australian population was split into two because we assume, based on previous studies, that these subpopulations have little to no gene flow between them (Hill *et al.* 1988, Higgins & Davis 1996). We conducted fieldwork in the 2004/2005 season at nesting colonies in the Southern Lagoon of New Caledonia (Baling *et al.* 2008, 2009), in the southern parts of Western Australia (hereafter 'Western Australia'), as well as in Tasmania, New South Wales and Victoria (hereafter the latter three Australian states are referred to as 'Eastern Australia') (Fig. 1a). We also obtained Australian samples from the Launceston Museum, Tasmania. New Zealand Fairy Tern samples comprise a mixture of bird carcasses (e.g. chicks), abandoned eggs and feather samples (originally acquired for DNA sexing) collected by the New Zealand Department of Conservation (DOC) together with those that were stored long-term at Victoria University of Wellington and Massey University (Supporting Information Table S1).

We conducted surveys in New Caledonia and Australia to search for Fairy Tern nesting colonies. At each colony, we first watched birds' behaviours and then collected feather samples from chicks, abandoned eggs (out of a nest) and freshly moulted feathers within nesting grounds. We captured adult Fairy Terns using metal cage traps within the nesting colonies. Once captured, we processed these birds (collected feather samples) outside of the colony to minimise disturbance to nesting birds. After release, the terns were monitored briefly to determine their condition and that they were exhibiting normal behaviour.

Laboratory methods

Genomic DNA was isolated from 0.5 mm of the calamus of each feather, extending up to the superior umbilicus (Horváth *et al.* 2005), using a total of two or three feathers per individual. We also used 0.2 g of muscle tissue of embryos or egg membranes from abandoned eggs. We only sampled one egg or chick from each clutch to avoid pseudo-replication. Samples were digested in 1.25 mm dithiothreitol (DTT) and 1.0 mg/mL proteinase K, and then subjected to standard phenol–chloroform extractions and ethanol precipitation.

We investigated the NADH dehydrogenase subunit 2 (ND2) region of the mitochondrial DNA genome using primers L5216 and H6313 (1097 bp) (Sorenson *et al.* 1999, Sorenson 2003). PCR amplifications were undertaken in a total volume of 50 μL: 1.5 units Platinum Taq Polymerase (Invitrogen, Carlsbad, CA, USA), the manufacturer's buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primer, and *c.* 100 ng genomic DNA. The PCR temperature profile consisted of one cycle of 94 °C for 7 min, 36 cycles of 94 °C for 15 s, 55 °C for 20 s and 72 °C for 1 min, followed by an extension of 72 °C for 10 min. The PCR products were run on a 1.6% agarose gel for size and concentration estimation. PCR products were purified using Exonuclease I/Shrimp Alkaline Phosphatase (GE Healthcare, Little Chalfont, UK), under profiles of 37 °C for 30 min and 80 °C for 15 min (Werle *et al.* 1994). Sequencing was performed in both directions using 1/8 reaction BigDye Terminator v3.1 (Applied Biosystems, Beverly, MA, USA) under basic ABI protocols. Reaction products were run on an ABI 3100 analyser.

Statistical analyses

Sequences for forward and reverse strands were examined and reconciled using Sequencher 4.2.4 (Genes Code Corp., Ann Arbor, MI, USA). Confirmation of species identity was conducted using BLAST (Benson *et al.* 2005) and compared with sequences from Bridge *et al.* (2005). We also compared the sequences and their translated proteins with available ND2 sequences of the same or other species to check for stop codons and to help ensure that we had not obtained nuclear copies of mitochondrial genes (NUMTs; Sorenson & Quinn 1998).

Multiple alignments of the sequences were performed using ClustalW (Thompson *et al.* 1994). We calculated basic genetic diversity among the populations using DnaSP 4.10 (Rozas *et al.* 2003). We then quantified within-population variation as haplotype diversity (H_d) and nucleotide diversity (π) in percentages, according to Nei (1987, eqn 10.6). Tajima's *D* test for neutrality was done for each population (Tajima 1989). Large significant *D*-values, either negative or positive, indicate a deviation from neutrality.

We constructed a minimum spanning network (MSN) in Arlequin v2.0 (Schneider *et al.* 2000) using the

haplotypes identified. Population differentiation (Φ_{ST}) was tested using an analysis of molecular variance (AMOVA; Excoffier *et al.* 1992), with Kimura 2-parameter distances (Kimura 1980). The significance of the fixation index (F_{ST}) was tested with 5000 permutations. We used pairwise Φ_{ST} to estimate the genetic distance between the populations. The average gene flow among the four populations was estimated using Φ_{ST} ($M = Nm$; Slatkin 1995) as implemented in Arlequin v2.0.

RESULTS

We collected 142 Fairy Tern samples in Australia and New Caledonia. The New Zealand samples obtained were collected from 1992 to 2005 ($n = 48$). We had a total of 86 sequences (1041 bp) that were good quality and identified as Fairy Terns, which were used in the final analyses (Supporting Information Table S2). Overall, we identified six haplotypes in the three countries (Fig. 1b). All haplotypes differed in transitions ($Ti = 10$) except for one that had a transversion substitution ($Tv = 1$, between haplotypes 1A and 5Z) (Supporting Information Table S3). Sequences were deposited in GenBank (Benson *et al.* 2005; Supporting Information Table S4).

Genetic structure and variation

There was only one haplotype observed within each of New Zealand, New Caledonia and Eastern Australia, and four haplotypes in Western Australia (Fig. 1b). Therefore, estimates of haplotype diversity, nucleotide diversity and their variances were zero within each group, with the exception of Western Australia ($H_d = 0.555$, $\pi = 0.08\%$). Polymorphism in Western Australia was consistent with neutral expectations and was not significant ($D = -0.337$). Tajima's D could not be calculated for Eastern Australia, New Caledonia or New Zealand due to the lack of genetic diversity within each population (Table 1).

The overall genetic structure among the Fairy Terns was high (91.41%, $\Phi_{ST} = 0.914$, AMOVA $P < 0.001$), which can be explained by the isolation of each breeding population from one another. New Zealand (haplotype 5Z) and New Caledonia (haplotype 6C) each consist of unique haplotypes (Fig. 1b). The difference between populations was very low (three to eight nucleotide substitutions) (Fig. 1b) but genetic differentiation of New Zealand Fairy Terns was still significant between Australia and New Zealand ($\Phi_{ST} = 0.91–1.00$, $P < 0.001$), and New Caledonia and Australia ($\Phi_{ST} = 0.84–1.0$, $P < 0.001$; Table 2). The most common haplotype, 1A, was observed in 52% of the Australian Fairy Tern individuals ($n = 86$) and was shared between the two subpopulations in Australia. The genetic distance was small between Western Australia and Eastern Australia subpopulations ($\Phi_{ST} = 0.087$; Table 2).

Gene flow between populations

The estimated gene flow (M) between the four locations was very low (Table 2), with the lowest calculated average of 0.05 migrants per generation between New Zealand and Western Australia. In contrast, the highest rate of gene flow was 5.25 between the two Australian subpopulations.

DISCUSSION

Overall, we found single unique haplotypes in New Zealand and New Caledonia, with shared haplotypes between the two subpopulations in Western and Eastern Australia. The Australian haplotype 1A had the highest number of mutational connections and therefore was presumed to be the oldest haplotype in this lineage (Crandall & Templeton 1993). The central position of A1 in the network suggests that the ancestors of the New Zealand Fairy Tern may have originated from the Australian population and later diverged. The network

Table 1. Estimates of within-population variability of the NADH 2 region of Fairy Tern from three populations: Australia (with two subpopulations; Eastern Australia and Western Australia), New Caledonia and New Zealand. The estimated population size (N_e) in 2002–2005, the total number of sequences (N_S), number of haplotypes (N_h), haplotype diversity (H_d), nucleotide diversity in percentage (π) and its associated variance (Var), and Tajima's D (D) within each of the populations.

				Haplotype diversity		Nucleotide diversity (%)		
Population	N_e	N_S	N_h	H_d	Var	π	Var	D
Eastern Australia	3000[a]	24	1	0.000	0.0000	0.000	0.000	N/A
Western Australia	6000[a]	31	4	0.555	0.0081	0.083	0.000	–0.337
New Zealand	30[a,b]	15	1	0.000	0.0000	0.000	0.000	N/A
New Caledonia	300[a]	16	1	0.000	0.0000	0.000	0.000	N/A
Overall	–	86	6	0.675	0.0017	0.003	0.000	0.728

[a]Wetlands International (2002, p. 202). [b]Ferreira *et al.* (2005).

Table 2. Population pairwise distances (Φ_{ST}) and estimation of migration rate ($M = Nm$) between Fairy Tern populations: Australia (subpopulations in Eastern Australia and Western Australia), New Caledonia and New Zealand. Above the diagonal and in bold: Φ_{ST}, $P < 0.001$**, $P < 0.01$*. Below diagonal: $M = Nm$.

	Australia			
Populations	Eastern Australia	Western Australia	New Zealand	New Caledonia
Eastern Australia	–	**0.087***	**1.000****	**1.000****
Western Australia	5.260	–	**0.913****	**0.837****
New Zealand	0.000	0.048	–	**1.000****
New Caledonia	0.000	0.098	0.000	–

structure we have recovered (Fig. 1) suggests that the New Caledonia population diverged from New Zealand. The lack of genetic overlap and the restricted migration rate among tern populations breeding in the three countries supports our hypothesis that the main breeding populations of Fairly Tern are not interbreeding.

Phylogeography

The phylogeographical patterns of colonially nesting seabirds within Australia and across greater distances typically show no structure across geographical distance (e.g. Austin *et al.* 1994, Friesen *et al.* 1996, Lombal *et al.* 2020). However, there are a few exceptions in some colonial seabird species (see Avise *et al.* 2000, Frugone *et al.* 2021), including the Fairy Tern. There are two potential reasons for the observed phylogeographical patterns of the Fairy Terns: historically, there was limited dispersal of Fairy Terns from Australia to New Zealand and New Caledonia, and these populations had varying degrees of isolation due to vicariance, and there was frequent migration (i.e. constant gene flow) between the three breeding populations in the past, although gene flow is now restricted due to anthropogenic factors (e.g. predation, human disturbance).

Our sampled Fairy Tern populations seem to encompass a structured phylogeographical pattern despite their relatively comparable geographical distances. Spatial distance between the two Australian subpopulations (Eastern Australia and Western Australia) is similar to distances from Eastern Australia to New Zealand and from New Zealand to New Caledonia populations. However, we did not observe any genetic structure between populations in Eastern Australia and Western Australia. Thus, it seems that the land connectivity in Australia supported gene flow between Eastern Australia and Western Australia subpopulations. Additionally, the Fairy Tern ancestor split from its closest relative, Little Tern *Sternula albifrons*, between 1 and 2 million years ago (Bridge *et al.* 2005), so it is likely that Fairy Terns would have had to reach New Zealand and New Caledonia across the sea. Thus, the ocean could pose a significant dispersal barrier.

Another possible explanation for the structured phylogeographical pattern is the difference in the availability of seasonal sources between the main populations. Fairy Terns are shallow-water feeders and therefore do not travel far from the breeding grounds to forage. They have been observed foraging in estuaries, sea wrack zones, reef flats and lagoons (Baling *et al.* 2009, Ismar *et al.* 2014, Dunlop 2018). The difference in availability of food sources among the breeding locations is at present unknown, but the nesting seasons vary in some populations (New Zealand: October–January; New Caledonia: June–October; Western Australia: October–January) (Baling *et al.* 2009, Ismar *et al.* 2014, Dunlop 2018, Greenwell *et al.* 2021). Populations that have different breeding times are less likely to interbreed with one another.

The New Zealand Fairy Tern has a long record of low population size and small numbers of breeding pairs, leading to some speculation that this population may have originally existed in low numbers (Parrish & Honnor 1997, Ferreira *et al.* 2005). This record matches the hypothesis of low occurrences of Fairy Terns arriving in New Zealand via the sea. However, we still unknown do not know the extent of genetic diversity of the New Zealand Fairy Tern pre-human arrival, whether the population originally had higher diversity (similar to the Australian population) and whether the effective population size has become reduced after the arrival of humans.

The New Caledonia Fairy Tern was expected to have higher genetic diversity due to having a considerably larger estimated population size than New Zealand (n = 100–200 breeding pairs, BirdLife International 2018) but, instead, the genetic diversity is similar to the New Zealand population. Pandolfi-Benoit & Bretagnolle (2002) described the New Caledonia Fairy Tern as on the 'verge of extinction'. The Tern was absent in the Southern Lagoon from 1993 to 1996 (Pandolfi-Benoit & Bretagnolle 2002) but nesting colonies were later found in 2004 (<25 breeding pairs, Baling *et al.* 2009). Additional colonies were discovered in the northwest of New Caledonia in 2018 (*c.* 50 pairs, Villard *et al.* 2020). The question of whether the overall New Caledonia population size has dramatically decreased over time or the colonies have moved outside of known survey areas needs further investigation.

Based on our current results, geographical distances and existing records of population size and tern

movement, we cannot choose between the scenarios of declining populations and persistence of small populations of Fairy Terns in New Zealand and New Caledonia. Specifically, we cannot determine whether these populations had larger genetic diversity in the past or have persisted with small effective population sizes. However, we do know that the current populations have distinct haplotypes specific to each country. Future detailed investigations using genome sequencing may be useful in constructing long-term demographic trends or a dated phylogenetic tree to tease out the effects of divergence due to anthropogenic vs. vicariance-induced causes (Vianna *et al.* 2020, Robinson *et al.* 2021).

Movement and migration

The estimated gene flow was restricted, meaning very low to no (matrilineal) interbreeding between the three main populations. Although gene flow between Western Australia and both New Zealand and New Caledonia was estimated to be low (0.048 and 0.098, respectively), these values could be a consequence of intrapopulation diversity calculated by Φ_{ST}. The lack of shared haplotypes suggests strongly that there is no female gene flow between New Zealand, New Caledonia and to either Australian region. This result is also congruent with the lack of records of Fairy Terns moving among the three populations during the breeding season. Although there are records of New Caledonia Fairy Terns in northeast Australia, there is no record of them staying to breed (Hill *et al.* 1988, Higgins & Davis 1996, Garnett & Crowley 2011). One exception was a sighting of a fledgling New Caledonian Fairy Tern begging food from an adult, which prompted suspicion of breeding in an islet at Australia's Coral Sea Territory (East Diamond Islet) *c.* 800 km from a known New Caledonian Fairy Tern breeding area (Carter & Mustoe 2007).

Strict philopatry is not often seen in seabirds (Serventy & Curry 1984). Although bird banding recoveries in Australia show that Fairy Terns generally return to historical breeding sites in the vicinity of their natal colony (see Higgins & Davis 1996), observers in some Australian states also described the terns as being seasonally migratory (Hill *et al.* 1988, Higgins & Davis 1996, Dunlop & Greenwell 2020). Hence, we expect a low degree of intermixing within the Australian population. Our study supports this hypothesis with the estimation of five migrants per generation between Western Australia and Eastern Australia. The estimated gene flow and low genetic differentiation within the two Australian subpopulations are also consistent with the lack of morphological divergence and degree of movement observed between Eastern and Western Australia Fairy Tern populations (see Higgins & Davis 1996). However, South Australia and the tern population situated in the northern part of Western Australia were not sampled in our study, and these populations may contain additional genetic variation.

Implications for New Zealand Fairy Tern conservation

The aim of conservation is not only to protect a population in its current range but also to conserve the genetic integrity of the whole species. The potential disappearance of small populations such as the New Zealand Fairy Tern could mean the loss of a portion of the evolutionary diversity within this species.

We recommend that the main populations (New Zealand, New Caledonia and Australia) continue to be considered as distinct breeding populations and continue to be managed as separate evolutionary significant units. Our results are consistent with current information on past genetic studies, described morphology, distribution and movements of Fairy Terns. Both the ND2 and cytochrome *b* (Chambers & Coddington 1998) loci showed low but significant genetic differentiation (i.e. one transversion nucleotide mutation) between New Zealand and the larger Australian population. Despite larger sample sizes, our study was limited to using one gene to investigate the matrilineal variation and movement between populations. Additionally, Chambers & Coddington (1998) concluded that the genetic distance between New Zealand and Australia Fairy Terns was less or equal to the distances to the species' closest relative, Little Tern. Incorporation of additional markers, particularly nuclear DNA (Moritz 1994) and genome sequencing will help increase the resolution of detailed movements among populations. Further investigations re-assessing the subspecies' taxonomic status should include quantitative comparisons between Fairy Tern genetic variation and their morphology.

We also strongly support the continuation of the New Zealand Fairy Tern as a single management unit. The low genetic diversity in these terns highlights the importance of reducing the external (i.e. predation and storms) and intrinsic (i.e. inbreeding depression) threats to the breeding population. The case of high infertility (33% of eggs) within this population (Ferreira *et al.* 2005, Hansen 2006) may be the result of inbreeding depression due to low effective population size. We suggest further genetic study to determine the degree and implications of inbreeding for the New Zealand population.

We express gratitude for all the help and support given by colleagues in New Zealand: Gwenda Pulham and Tony Habraken (OSNZ); Rosalie Stamp, Katrina Hansen and Tony Beaucamp (DOC); Geoff Chambers (Victoria University of Wellington); Michael Anderson, Jo Peace, Luis Ortiz Catedral, Weihong Ji, David Lambert and Robin Fordham (Massey University); Sally Aitken (MAF); Don Love (UoA AEC); Ngaire White

(Uniservices Ltd); Debbie Steel, Darryl Jeffries, Emma Marks, Shane Lavery, Stuart Parsons and the ecology labs in both University of Auckland and Massey University; in New Caledonia: Nicolas Barré & Jérôme Spaggiari (SCO); François Devinck, Benoit Perignon, Sven Nenu, Christophe Gosset and his crew of the *Isabelle*, and Federic Paul and his crew (DRN – *Province Sud*); Gavin Hunt; and Pascal Villard; and in Australia: Nic Dunlop; Mike Bamford; Peter Collins; Pricilla Park; Sue & Col Dyke; Peter McGlone; Harriet Davie (Rottnest Island Authority); Jane Prince (University of WA); Adrian Coleman, Peter Mawson and Peter Lambert (WA CALM); Joanne Wann (Dept of Defense); Sally Bryant (TAS DPIWE); Clive Minton (AWSG); Jonathan Stevens, Faye Bedford and Sue Hadden (VIC DSE); Jillian Keating and Roslyn Barr (NSW NPWS); the Swan River Trust; Melville City Council; and Launceston Museum. A final thank you for the support given by the School of Biological Sciences of the University of Auckland during the course of this project. Open access publishing facilitated by The University of Auckland, as part of the Wiley – The University of Aucklandagreement via the Council of Australian University Librarians

FUNDING

The project was funded by the New Zealand Department of Conservation.

ETHICAL NOTE

Methods of sample collection were approved by the University of Auckland Animal Ethics Committee (AEC/02/2004/R225) and the Animal Care and Ethics Committee of the Director-General of NSW Department of Primary Industries (No. 04/4884). Additional research permits were obtained from the NZ Department of Conservation (DOC, AK/13723/RES); the *NC Province Sud of Direction des Ressources Naturelles* (No. 6024-3429 DRN/ENV); the Western Australian Department of Conservation and Land Management (CALM, No. SF004778 & No. CE000830); Rottnest Island Authority (No. 2004/028); Tasmania Department of Primary Industries, Water and Environment (DPIWE, No. TFA 04195); the Victoria Department of Sustainability and Environment (DSE, No. 10002223); and the New South Wales National Park and Wildlife Service (NPWS, No. S11455).

AUTHOR CONTRIBUTION

Marleen Baling: Data curation (lead); Formal analysis (lead); Funding acquisition (supporting); Investigation (lead); Methodology (lead); Project administration (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review & editing (lead). **Dianne Brunton:** Conceptualization (lead); Funding acquisition (lead); Project administration (supporting); Resources (lead); Supervision (lead); Writing – review & editing (supporting).

Data Availability Statement

Data are available in the Supplementary Online Information and GenBank (MW371124–MW371210).

REFERENCES

Austin, J.J., White, R.W.G. & Ovenden, J.R. 1994. Population-genetic structure of a philopatric, colonially nesting seabird, the Short-tailed Shearwater (*Puffinus tenuirostris*). *Auk* **111**: 70–79.

Avise, J.C., Nelson, W.S., Bowen, B.W. & Walker, D. 2000. Phylogeography of colonially nesting seabirds, with special reference to global matrilineal patterns in the Sooty Tern (*Sterna fuscata*). *Mol. Ecol.* **9**: 1783–1792.

Baling, M., Brunton, D.H. & Jeffries, D. 2008. Marine and coastal bird survey of islands in the Southern Lagoon, New Caledonia. *Notornis* **55**: 111–113.

Baling, M., Jeffries, D., Barré, N. & Brunton, D.H. 2009. A survey of Fairy Tern (*Sterna nereis*) breeding colonies in the Southern Lagoon, New Caledonia. *Emu* **109**: 57–61.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Wheeler, D.L. 2005. GenBank. *Nucleic Acids Res.* **33**: D33–38.

BirdLife International. 2018. *Sternula nereis*. **Vol. 2018**, pp. e.T22694691A132568135. The IUCN Red List of Threatened Species.

Borsa, P., Pandolfi, M., Andréfouët, S. & Bretagnolle, V. 2010. Breeding avifauna of the Chesterfield Islands, Coral Sea: current population sizes, trends, and threats. *Pac. Sci.* **64**: 297–314.

Bridge, E.S., Jones, A.W. & Baker, A.J. 2005. A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. *Mol. Phylogenet. Evol.* **35**: 459–469.

Carter, M. & Mustoe, S. 2007. Another form of Fairy Tern *Sterna nereis* breeding in Australian Territory. *Aust. Field Ornithol.* **24**: 167–179.

Chambers, G.K. & Coddington, S.J. 1998. Molecular systematics of New Zealand Fairy Tern (*Sterna nereis davisae*) based on mitochondrial DNA sequences. In: *Conservation advisory science notes*: 1–9. Wellington: Department of Conservation.

Coddington, S. 1996. Genetic variation of three New Zealand bird subspecies: Fernbirds (*Bowdleria punctata*), New Zealand Pigeon (*Hemiphaga novaseelandiae*), Fairy Terns (*Sterna nereis*). Wellington: School of Biological Sciences, Victoria University of Wellington, pp. 85.

Crandall, K. & Templeton, A.R. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* **134**: 959–969.

Dunlop, J.N. 2018. *Fairy Tern (Sternula nereis*) Conservation in South-Western Australia. West Perth: Conservation Council of Western Australia.

Dunlop, J.N. & Greenwell, C.N. 2020. Seasonal movements and metapopulation structure of the Australian Fairy Tern in Western Australia. *Pac. Conserv. Biol.* **27**: 47–60.

Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

Falla, R.A., Sibson, R.B. & Turbott, E.G. 1979. *A new Field Guide to the Birds of New Zealand*. Auckland: Collins.

Ferreira, S.M., Hansen, K.M., Parrish, G.R., Pierce, R.J., Pulham, G.A. & Taylor, S. 2005. Conservation of the endangered New Zealand Fairy Tern. *Biol. Cons.* **125**: 345–354.

Friesen, V.L., Montevecchi, W.A., Baker, A.J., Barretts, R.T. & Davisdson, W.S. 1996. Population differentiation and evolution in the Common Guillemot *Uria aalge*. *Mol. Ecol.* **5**: 793–805.

Frugone, M.J., Cole, T.L., López, M.E., Clucas, G., Matos-Maraví, P., Lois, N.A., Pistorius, P., Bonadonna, F., Trathan, P., Polanowski, A., Wienecke, B., Raya-Rey, A., Pütz, K., Steinfurth, A., Bi, K., Wang-Claypool, C.Y., Waters, J.M., Bowie, R.C.K., Poulin, E. & Vianna, J.A. 2021. Taxonomy based on limited genomic markers may underestimate species diversity of Rockhopper Penguins and threaten their conservation. *Divers. Distrib.* **27**: 2277–2296.

Garnett, S.T., Szabo, J.K., Dutson, G. & Ehmke, G. 2011. Fairy Tern (New Caledonian). In *The Action Plan for Australian Birds 2010*. Collingwood, Victoria: CSIRO Publishing, pp. 465–467.

Greenwell, C.N., Dunlop, J.N., Admiraal, R. & Loneragan, N.R. 2021. The secret life of Fairy Terns: breeding chronology and life history observations of *Sternula nereis nereis* in south-western Australia. *Pac. Conserv. Biol.* **27**: 143–154.

Hansen, K. 2006. *New Zealand FAIRY TERN (Sterna nereis davisae*) Recovery Plan, 2005–15. Wellington: Science & Technical Publishing.

Higgins, P.J. & Davis, S.J.J.F. 1996. Fairy tern. In Higgins, P.J. & Davis, S.J.J.F. eds., *Handbook of Australian, New Zealand and Antarctic Birds, Volume 3: Snipe to Pigeons*. Vol. 3. Melbourne: Oxford University Press, pp. 384–385.

Hill, R., Bamford, M., Rounsevell, D. & Vincent, J. 1988. *Little Terns and Fairy Terns in Australia – a RAOU Conservation Statement*. Moonee Ponds: Royal Australasian Ornithologists Union (RAOU), Publication No. VRH 7064.

Hitchcock, W.B. 1959. A review of the 'least' tern in Australian waters. *South Aust. Ornithol.* **22**: 87–106.

Hitchcock, W.B. 1967. Bird in the hand - Little Tern and Fairy Tern. *Aust. Bird Band.* **5**: 14.

Horváth, M., Martínez-Cruz, B., Negro, J.J., Kalmár, L. & Godoy, J.A. 2005. An overlooked DNA source for non-invasive genetic analysis in birds. *J. Avian Biol.* **36**: 84–88.

Ismar, S.M.H., Trnski, T.O.M., Beauchamp, T., Bury, S.J., Wilson, D., Kannemeyer, R., Bellingham, M. & Baird, K. 2014. Foraging ecology and choice of feeding habitat in the New Zealand Fairy Tern *Sternula nereis davisae*. *Bird Conserv. Int.* **24**: 72–87.

Kimura, M. 1980. A simple method for estimating evolutionary rate base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111–120.

Kinsky, F.C. 1970. *Annotated Checklist of the Birds of New Zealand*. Wellington: A.H. & A.W. Reed.

Lombal, A.J., O'dwyer, J.E., Friesen, V., Woehler, E.J. & Burridge, C.P. 2020. Identifying mechanisms of genetic differentiation among populations in Vagile species: historical factors dominate genetic differentiation in seabirds. *Biol. Rev.* **95**: 625–651.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. *Mol. Ecol.* **3**: 401–411.

Nei, M. 1987 *Molecular Evolutionary Genetics*. New York: Columbia University Press.

Pandolfi-Benoit, M. & Bretagnolle, V. 2002. Seabirds of the Southern Lagoon of New Caledonia, distribution, abundance and threats. *Waterbirds* **25**: 202–213.

Parrish, R. & Honnor, L. 1997. *New Zealand Fairy Tern (Tara-iti) Sterna nereis davisae* Recovery Plan 1997–2002. Wellington: Department of Conservation.

Robertson, H.A., Baird, K., Dowding, J.E., Elliott, G.P., Hitchmough, R.A., Miskelly, C.M., McArthur, N., O'Donnell, C.F.J., Sagar, P.M., Scofield, R.P. & Taylor, G.A. 2017. *Conservation Status of New Zealand Birds, 2016*. Wellington: Department of Conservation.

Robinson, J.A., Bowie, R.C.K., Dudchenko, O., Aiden, E.L., Hendrickson, S.L., Steiner, C.C., Ryder, O.A., Mindell, D.P. & Wall, J.D. 2021. Genome-wide diversity in the California Condor tracks its prehistoric abundance and decline. *Curr. Biol.* **31**: 2939–2946.e5.

Rozas, J., Sánchez-Delbarrio, J.C., Messeguer, X. & Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**: 2496–2497.

Schneider, S., Roessli, D. & Excoffier, L. 2000. *Arlequin Version 2.000: A Software for Population Genetics Data Analysis*. Geneva: Genetics and Biometry Laboratory, University of Geneva.

Serventy, D.L. & Curry, P.J. 1984. Observations on colony size, breeding success, recruitment and intercolony dispersal in a Tasmanian colony of Short-tailed Shearwaters *Puffinus tenuirostris* over a 30-year period. *Emu* **84**: 71–79.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**: 457–462.

Sorenson, M.D. 2003. *Avian mtDNA Primers*. Boston: Boston University.

Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T. & Mindell, D.P. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Mol. Phylogenet. Evol.* **12**: 105–114.

Sorenson, M.D. & Quinn, T.W. 1998. Numts: a challenge for avian systematics and population biology. *Auk* **115**: 214–221.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585–595.

Tarburton, M.K. 2001. Observations on the status of the land birds, wading birds and seabirds of Samoa. *Emu* **101**: 349–360.

Taylor, G., Hansen, K. & Ferreira, S. 2004. *Technical Review of the New Zealand Fairy Tern Recovery Programme*. Wellington: Department of Conservation.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680.

Vianna, J.A., Fernandes, F.A.N., Frugone, M.J., Figueiró, H.V., Pertierra, L.R., Noll, D., Bi, Ke, Wang-Claypool, C.Y., Lowther, A., Parker, P., Le Bohec, C., Bonadonna, F., Wienecke, B., Pistorius, P., Steinfurth, A., Burridge,

C.P., Dantas, G.P.M., Poulin, E., Simison, W.B., Henderson, J., Eizirik, E., Nery, M.F. & Bowie, R.C.K. 2020. Genome-wide analyses reveal drivers of penguin diversification. *Proc. Natl Acad. Sci. USA* **117**: 22303–22310.

Villard, P., Hunt, G., Coll, J. & Cassan, J.-J. 2020. Breeding biology of Fairy Terns on Magone Islet, New Caledonia. *Emu* **120**: 360–364.

Werle, E., Schneider, C.J., Renner, M., Völker, M. & Fiehn, W. 1994. Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Res.* **22**: 4354–4355.

Wetlands International. 2002. Waterbird population estimates. In: *Wetlands International Global Series no. 12*. Wageningen, The Netherlands: Wetlands International, pp. 1–226.

Received 13 April 2021;
Revision 1 July 2021;
revision accepted 18 January 2022.
Associate Editor: Jon Martin Collinson.

SUPPORTING INFORMATION

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

Table S1. List of New Zealand Fairy Tern samples acquired from New Zealand Department of Conservation, Massey University and Victoria University of Wellington. Remaining samples are stored at Unitec Institute of Technology.

Table S2. List of Fairy Tern samples from New Zealand (n = 48), New Caledonia (n = 41) and Australia (n = 102).

Table S3. Distribution of the six observed ND2 region haplotypes (n = 86) of Fairy Tern from three breeding populations: Australia (subpopulations of southeast Australia, Western Australia), New Caledonia and New Zealand.

Table S4. List of Fairy Tern sequences from New Zealand (n = 15), New Caledonia (n = 16) and Australia (n = 55).