EMU AUSTRAL ORNITHOLOGY

Emu - Austral Ornithology

ISSN: 0158-4197 (Print) 1448-5540 (Online) Journal homepage: https://www.tandfonline.com/loi/temu20

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**To cite this article:** Miles V. Keighley, Robert Heinsohn, Naomi E. Langmore, Stephen A. Murphy & Joshua V. Peñalba (2019) Genomic population structure aligns with vocal dialects in Palm Cockatoos (*Probosciger aterrimus*); evidence for refugial late-Quaternary distribution?, Emu - Austral Ornithology, 119:1, 24-37, DOI: 10.1080/01584197.2018.1483731

**To link to this article:** https://doi.org/10.1080/01584197.2018.1483731

EMU - AUSTRAL ORNITHOLOGY
2019, VOL. 119, NO. 1, 24–37
https://doi.org/10.1080/01584197.2018.1483731

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# Genomic population structure aligns with vocal dialects in Palm Cockatoos (*Probosciger aterrimus*); evidence for refugial late-Quaternary distribution?

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**ABSTRACT**

Species persistence and maintenance of genetic diversity are strongly affected by dispersal and historical distribution, especially when species depend on habitat that is non-uniform or fluctuates dramatically with changing climate. Australo-Papuan rainforest has fluctuated dramatically since the last glacial maximum (around 20 kya). To understand how prehistoric climate fluctuation affected population connectivity and genetic diversity in a rainforest edge species, we screened 27 Palm Cockatoo samples from Cape York Peninsula (Australia) and southern Papua New Guinea (PNG) in 1132 single nucleotide polymorphisms in 342 nuclear loci and the mitochondrial *ND2* gene. We also modelled the birds' distribution at present, mid-Holocene (~6 kya) and the last glacial maximum (~21 kya). Population differentiation in nuclear genomic data among Australian populations aligns with distribution contraction to mountainous refugia at the mid-Holocene (~6 kya). Lack of nuclear divergence between PNG and Australia may reflect late-Holocene recolonisation, but different *ND2* haplotypes suggest early stages of divergence. Although admixed individuals suggest some gene flow, recent movement restriction to/from Australian refugia is suggested by a unique *ND2* haplotype, genomic divergence and a vocal dialect boundary shown previously. Our results show how prehistoric climate fluctuation affects present-day and future species conservation in dynamic rainforest edge ecosystems.

**ARTICLE HISTORY**
Received 29 August 2017
Accepted 28 May 2018

**KEYWORDS**
Australia; conservation biogeography; distribution model; Iron Range; New Guinea; parrot; Palm Cockatoo; *Probosciger*

## Introduction

Understanding the contemporary structure of wild avian populations has great value for informing species conservation, especially where species distributions are non-uniform or fragmented throughout the landscape (Pavlova *et al.* 2012). Fragmentation of species distributions, either naturally (through past and present biogeographical influences) or human-induced (through land use, e.g. vegetation clearing for agriculture), creates separate, small populations which are more vulnerable to local decline from local threats or stochastic fluctuations in population size (Frankham 2005). Population declines can cause loss of genetic and cultural diversity resulting in lower adaptability to change, consequently increasing the species' vulnerability to extinction (Frankham 2005). However, local declines can be buffered with replenishment by immigration or gene flow from connected populations, which convey the benefits of increased effective population sizes and lower extinction vulnerability in small populations (Frankham 2005; Sunnucks 2011). These dynamics are important in determining which species or populations of species recover or disappear following dramatic changes in distribution due to climate or disturbance.

The present-day distribution of rainforest in the Australia-New Guinea region reflects dramatic worldwide climate and sea-level changes during the Pleistocene (1.6 mya–10 ka) creating complex biogeographical histories for many species. A land bridge between the two landmasses was exposed for most of the past 250 ka (Chappell and Shackleton 1986) resulting in the rainforests of Cape York Peninsula, northeastern Queensland, sharing greater floral (Webb and Tracey 1981; Barlow and Hyland 1988; Crisp *et al.* 2001) and faunal (Kikkawa *et al.* 1981) similarity with New Guinean rainforests than other rainforests within Australia. Global climate change in the recent past has caused these rainforest patches to contract greatly (Nix

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The underlying research materials for this article can be accessed by contacting the corresponding author.

This article was originally published with errors. This version has been corrected. Please see Erratum (http://dx.doi.org/10.1080/01584197.2018.1513308).

Supplemental data for this article can be accessed here.

and Kalma 1972), making it unclear how population-level dynamics of rainforest species have been affected. Dynamics such as where they persisted or recolonised following re-expansion of refugial rainforests and how populations are connected in the present are even less clear for species occupying the ecotone between rainforest and woodland (e.g. Sooty Owls (*Tyto tenebricosa*); Norman *et al.* 2002).

The rainforest edge species, the Palm Cockatoo (*Probosciger aterrimus*), is ideal for investigating the effects of dynamic historical distribution because information about their population structure is also urgently required to forecast the rate of future population declines and predicting viability (Murphy *et al.* 2003). Palm Cockatoo populations in eastern New Guinea and Australia have not diverged sufficiently to be considered separate subspecies, yet their aversion to crossing open water (Igag 2002; Murphy *et al.* 2003) separates these populations for conservation purposes. In Australia, Palm Cockatoos have low breeding success; on average, each pair lays just one egg every 2 years and only 20% of their eggs result in a fledgling (Murphy *et al.* 2003). Moreover, they are threatened with habitat loss both from changing fire regimes and vegetation clearing due to mining activity in the west of Cape York Peninsula, northern Australia (Murphy *et al.* 2003; Heinsohn *et al.* 2009). Globally, Palm Cockatoos are 'least concern' (BirdLife International 2016); however, the 'vulnerable' status of Australian Palm Cockatoos is owed to an unsubstantiated estimate of between 2500 and 10 000 individuals assumed to form a single panmictic subpopulation, as well as predicted declines with a probability of extinction greater than 10% in the next 100 years (Heinsohn *et al.* 2009; IUCN criteria C and E, Department of the Environment 2015). The assumption that Australian Palm Cockatoos form a single subpopulation may be inaccurate, given that they occur in fragmented rainforest patches and poor connectivity between patches may restrict gene flow.

Palm Cockatoos breed in monogamous pairs which defend nests in hollow trees within 1 km of rainforest where they feed (Murphy *et al.* 2003). Rainforest adheres to drainage patterns and patches of suitable soil or topography among sclerophyll woodland (Webb and Tracey 1981). The extent to which subpopulations of Palm Cockatoos are interconnected on Cape York Peninsula could profoundly influence their persistence because some may act as sinks and others as sources (Diffendorfer 1998). Whether discontinuous rainforest patches allow connectivity among separate subpopulations is unknown, though disrupted connectivity is possible given spatial differentiation of cultural characteristics among Australian Palm Cockatoos (Keighley *et al.* 2016).

Palm Cockatoos conduct unique displays including postures, gestures, and the use of a manufactured sound tool to 'drum' on their nest hollow (Wood 1984). Although the drumming behaviour is widespread within Cape York Peninsula (pers. obs.), Palm Cockatoos from the Iron and McIlwraith Ranges region (considered one coherent population due to habitat continuity, hereafter 'Iron Range') appear to use sound tools more frequently (Heinsohn pers. comm.), and have a unique vocal dialect in the contact call compared to other Cape York populations (Figure 1(a)) (Keighley *et al.* 2016). These cultural differences could reflect disruption in past or current connectivity between Iron Range and other populations preventing diffusion of cultural behaviour and allowing for cultural divergence, or the differences could reflect assimilation of local cultures by mobile individuals (Freeberg 2000; Podos and Warren 2007). Three possible explanations for evolution of cultural differences are (1) contemporary separation with behaviour currently undergoing divergence, (2) historical separation in refugial rainforest patches with differences maintained by learning despite ongoing connectivity, or (3) that they are purely an artefact of learning without separation. Identifying the basis for potential connectivity disruption is crucial for conservation management and increasing understanding of interaction between cultural diversity and population-level processes. To provide additional data about historical and recent population dynamics we examine variation in one mitochondrial gene and nuclear genome-wide single nucleotide polymorphisms (SNPs) of Palm Cockatoos from Australia and southern Papua New Guinea. To investigate potential biogeographical drivers for population structure we compare our genomic data with present and past distribution models.

## Methods

### *Sampling*

We used a total of 27 Palm Cockatoo samples for genetic screening (sample details in supplemental material Table S1). Of these, 22 samples were from Cape York Peninsula, Australia, including five from the Iron Range, and five were from Papua New Guinea (Figure 1(b)). The DNA from the Iron Range population was all extracted from blood samples using the Qiagen Blood and Tissue DNeasy kit (Qiagen, Valencia, CA, USA). The samples from Bamaga on Cape York Peninsula, Australia and the remainder of Papua New Guinea were extracted from

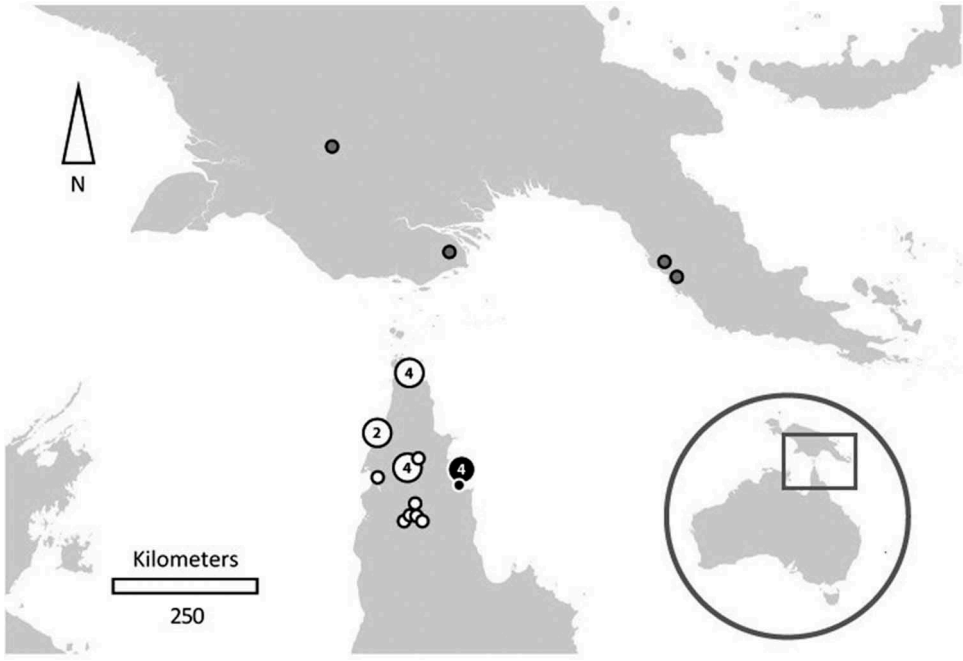


Figure 1a. Geographic sampling. The map depicts the geographic sampling of the Palm Cockatoo populations in Australia and Papua New Guinea. Black circles indicate samples from the Iron Range, white circles indicate samples from the rest of Cape York Peninsula, and grey circles indicate samples from Papua New Guinea. Where samples are clustered together, numbers inside circles indicate their abundance whereas circles otherwise represent single samples.

museum toe-pad samples. The blood and toe-pad samples were obtained and DNA was extracted for a previous project and the details of the extraction procedure are outlined in (Murphy *et al.* 2007). The remaining DNA samples were extracted non-invasively from moulted feathers collected throughout the range of the Cape York population. Apart from one feather from a captive individual at Weipa (western Cape York Peninsula), samples were collected from the ground by M.V.K. and volunteer assistants or contributed with location data by third-party individuals. Collection locations were at least 2 km apart, which likely exceeds the range of an individual's territory (Murphy *et al.* 2003). The extraction method used is as described in Horváth *et al.* (2005). The feathers were extracted using a Qiagen DNeasy Kit. From flight feathers, we took a ~8 × 5 mm portion of the quill adjacent to where the barbs end. From contour feathers we used the entire calamus. The samples were digested in 180 µL ATL Buffer, 20 µL ProK, and 10 µL 1 M dithiothrietol at 65°C overnight. Following the remaining steps of the standard Qiagen protocol, the samples were eluted in two sets of 150 µL AE Buffer and concentrated using a SpeedVac.

## DNA sequencing

A summary of our methods for molecular sequencing, data processing and calculation of population structure and statistics is presented here, with full details in supplemental material. We used a modified version of the 'hyRAD' protocol (Suchan *et al.* 2016) to screen the nuclear genome for anonymous SNPs. The protocol combines the efficiency of restriction-associated DNA sequencing (RADseq) with the accuracy and power of hybridisation capture methods, allowing utilisation of degraded DNA from moulted feathers or historically sampled museum specimens. The hyRAD protocol uses double-digest restriction digest (ddRAD) sequencing libraries as DNA probes for a sequence capture (Peterson *et al.* 2012; Suchan *et al.* 2016).

Probes for the hyRAD protocol were designed from ddRAD libraries of the four Iron Range samples due to their high DNA quality following a protocol similar to Peñalba *et al.* (2014). The Iron Range ddRAD libraries were built with restriction enzymes PstI and EcoRI (Peterson *et al.* 2012) and fragments were size selected to 345–407 bp. After probe design, the ddRAD Iron Range libraries were sequenced along with captured hyRAD libraries of all other sites as paired-end reads using an Illumina high-throughput NextSeq500 at the ACRF Biomolecular Resource Facility.

Whole mitochondrial genomes were obtained as a by-product of hyRAD capture except for samples from the Iron Range, which were not included in the capture. For the Iron Range samples, the mitochondrial *ND2* (NADH dehydrogenase subunit 2) gene was

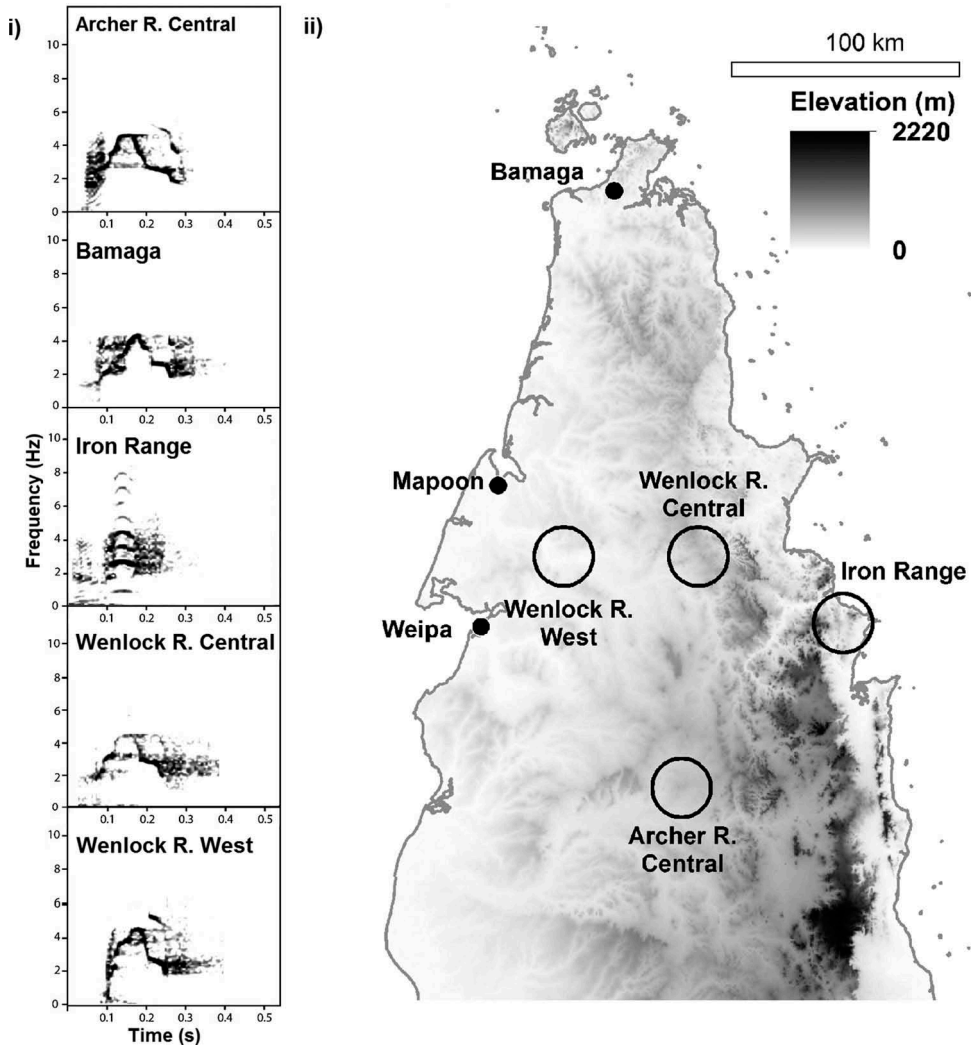


Figure 1b. Contact calls and elevation. (i) Spectrograms of representative contact calls from five locations on Cape York Peninsula marked on the elevation map in (ii). Note: spectrograms were created in RavenPro v.1.5 (Charif et al. 2008) (16-bit sample format; frame overlap = 50%; Hann Window, DFT = 512; frequency resolution = 124 Hz).

amplified with primers L5204 and H6312 (Sorenson *et al.* 1999). The *ND2* marker was chosen to complement previous mitochondrial studies (Murphy *et al.* 2007) and due to its relatively fast mutation rate (Pacheco *et al.* 2011). In total, 838 bp of the *ND2* gene were sequenced.

### Data processing

The hyRAD- and ddRAD-derived data sets were filtered using existing and custom pipelines. In particular, ddRAD data were filtered for low-complexity reads and both hyRAD and ddRAD were trimmed for low-quality sites, barcodes and restriction cut sites. A nuclear sequence reference panel was assembled from the cleaned Iron Range ddRAD data. This reference contig set was used to map all the individuals. Genotype likelihood calculations (suited to low-coverage data) (Nielsen *et al.* 2011, 2012) and SNP filtering based on coverage, overlap between populations or putative repeats and paralogous regions were conducted with ANGSD (Korneliussen *et al.* 2014). Unlinked SNPs were used for population structure and all SNPs within all loci were carried through for the other population genetic statistics. A *de novo* assembly of the mitochondrial genome was obtained using four samples, with subsequent mapping of cleaned reads. *ND2* sequences were then extracted from the whole mitochondrial genome assembly, aligned with the Sanger sequenced *ND2* sequences, and inspected for population structure and haplotype diversity.

### Population structure and statistics

We recovered the nuclear genome population structure using the ngsTools kit (Fumagalli *et al.* 2014; Vieira *et al.* 2016) using genotype likelihoods. The output

from ngsTools was used to create a network in SplitsTree (Dress *et al.* 1996) and to summarise distance information using multidimensional scaling (MDS). The mitochondrial *ND2* population structure was visualised using haplotype networks in PopArt (Leigh and Bryant 2015). To try to detect additional population structure and estimate admixture between populations we used ngsAdmix (Skotte *et al.* 2013). Formal methods of analysis are available for gene flow but these are likely to lead to inaccurate and unreliable estimates given the current state of sampling. Estimates of gene flow were therefore not included to maintain the conservative nature of claims made. We discuss these analyses and our data further in the supplemental material.

Population genetics summary statistics were estimated from allele frequencies that were derived from genotype likelihoods. These statistics included an estimation of population differentiation ($F_{ST}$; Reynolds *et al.* 1983), population divergence ($d_{xy}$), per site heterozygosity ($\theta$), per site nucleotide diversity ($\pi$) and divergence after population split ($D_A$).

### Species distribution modelling

To determine whether the observed genetic population structure had historical or contemporary origins we modelled Palm Cockatoo distributions using environmental data from the present, the mid-Holocene (~6 kya) and the last glacial maximum (~21 kya) from the WorldClim database (http://worldclim.com). In these databases, past climate was obtained by downscaling and calibrating based on present data (http://www.worldclim.org/downscaling). To acquire environmental data representative of Palm Cockatoo habitat requirements, we used data from the present climate database at geographic locations of museum voucher occurrence points from the Atlas of Living Australia Database (http://www.ala.org.au – accessed 12 October 2016). Spurious localities and duplicate points were removed and to compensate for bias due to sampling effort we used only one occurrence point in a 0.5 degree cell. After filtering the occurrence points, we had 37 occurrence points remaining for our model training and testing (coordinates provided in supplemental material Table S2). To ensure parsimonious distribution models we removed covariable bioclimatic variables (Spearman Rank Correlation test $\rho \leq 0.7$) in the cells containing the occurrence points (available at: http://www.worldclim.org/bioclim). We were left with temperature seasonality (BIO4), minimum temperature of coldest month (BIO6), mean temperature of wettest quarter (BIO8), precipitation of wettest quarter (BIO16), and precipitation of warmest quarter (BIO18) as our predictor variables. We used both BIOCLIM and MAXENT models to predict the species range (Phillips *et al.* 2006; Booth *et al.* 2014). BIOCLIM models are simple to implement and describe the *n*-dimensional climatic dataspace in terms of simple ranges but they do not account well for interactions between predictors. MAXENT models develop a response curve for each environmental variable, indicating which particular conditions within a range are most suitable (Booth *et al.* 2014). Both methods use presence-only data, which has its own limitations (Elith 2011). We generated 5000 background points within the extent 135, 150, −18, −5. We used 80% of the occurrence and background points to train the model and the remaining 20% to test the model. To evaluate the models we calculated the area under the receiver-operator curve (AUC) by averaging out the AUC values for each 80% training data subset. We also calculated a null model calibrated AUC (cAUC) value with the spatial sorting bias (SSB) removed as the background extent may affect the initial AUC calculation. Models with an AUC value of >0.7 tend to be regarded as informative, though the same models tend to have a cAUC value of ~0.5 after removing SSB (Hijmans 2012).

## Results

### Population structure and admixture

After rigorous SNP filtering we recovered 342 contigs with 1132 SNPs in a total of 83 093 bp. A detailed summary of the recovered reads and RAD loci per sample can be found in supplemental material Table S3. For the nuclear SNP loci, the Iron Range population comes out as a separate population from the remaining Cape York and Papua New Guinea samples. This is evident in the network (Figure 2(a)) and in the MDS plot where the first dimension separating the population contains 21.88% of the variation (Figure 2(b)). The *ND2* network shows only three haplotypes, one representing all Papua New Guinea samples, one containing samples from Cape York Peninsula and the Iron Range, and one unique haplotype in the Iron Range (ANU19, Figure 2(c)). The *ND2* haplotypes have a maximum divergence of two mutations. One sample from Iron

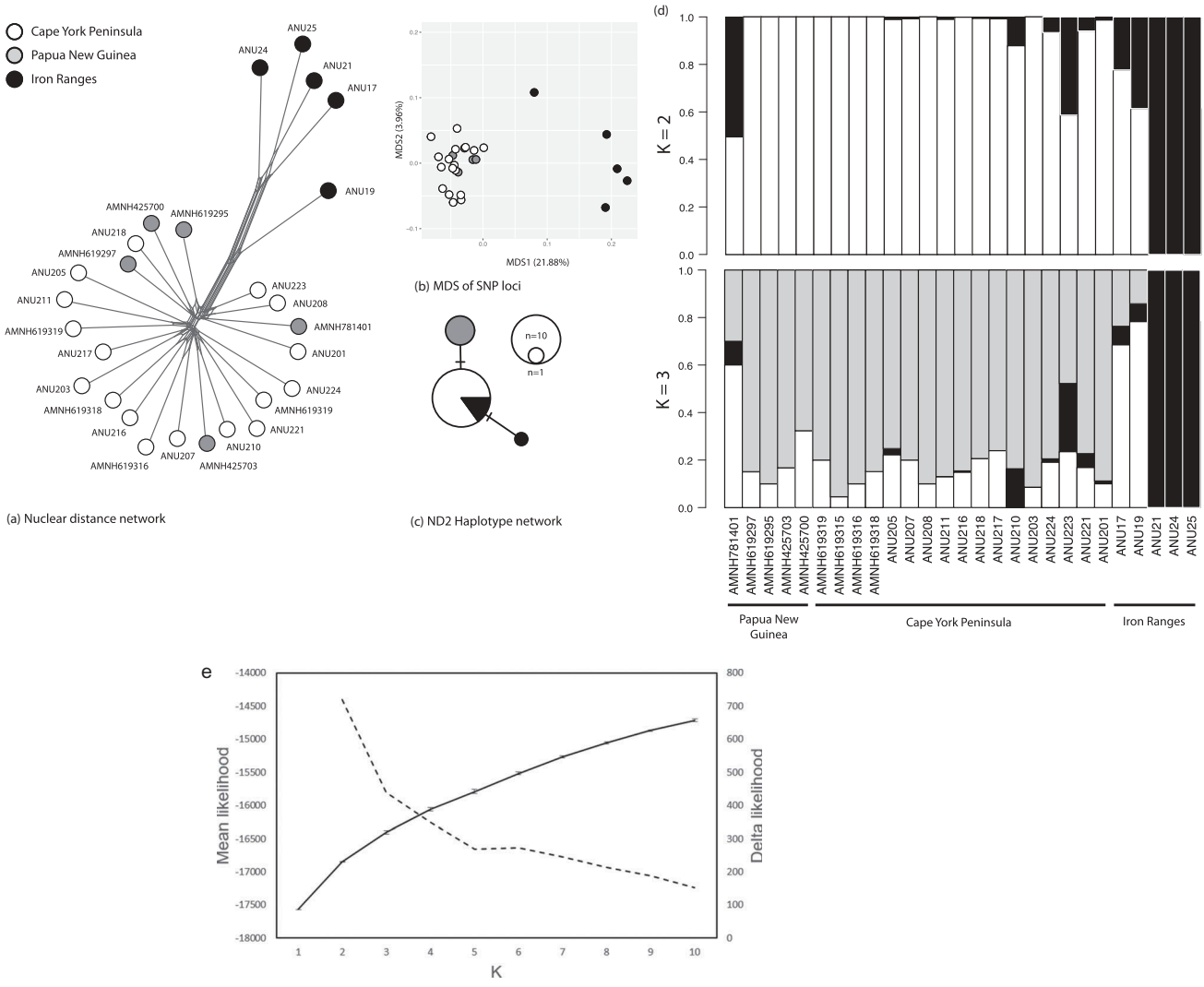


**Figure 2.** Population structure. Each plot represents the population structure, with the colours of the samples corresponding to the sample map. (a) Represents a network of the distance matrix derived from genotype likelihoods of the nuclear SNPs between each sample. (b) Represents a multidimensional scaling plot also derived from the distance matrix from the genotype likelihoods. (c) Shows a haplotype network of a segment of the mitochondrial *ND2* locus. (d) Represents our results from the admixture analysis for two clusters (K = 2) and three clusters (K = 3). The change in mean (delta) likelihood by increasing the number of clusters for each value of K from 2 to 5 is shown in (e) (solid line: mean log likelihood ± SD in 10 repetitions for each number of clusters; dashed line: delta likelihood with increasing K).

Range that was sequenced using both the ddRAD and hyRAD protocols consistently fell in the same population, providing evidence that the population structure is biological and not due to the sequencing method.

The results of our admixture analysis show the increase in (delta) likelihood is much greater at K = 2, than with subsequent K values, and division into three clusters adds little information (Figure 2(e)), therefore we discuss the structure shown by analyses at K = 2. The analysis shows that admixed individuals within the Iron/McIlwraith Ranges and the Cape York Peninsula populations (Figure 2(d)) provide preliminary evidence for gene flow. Admixed individuals from Cape York Peninsula occur primarily west, but also south-west, of the Iron Range population (Figure 3). Inconsistencies between the population structure from the distance matrix and from the admixture analyses may be due to the difference in robustness of these analyses to missing data. Individuals outside of the Iron Range with the highest amounts of admixture (ANU223 and AMNH781401) also tend to have the highest amount of missing data (supplemental Table S3, Figures 2 and 3). On the other hand, the evidence for admixture in ANU19 from the Iron Range is supported by both the distance matrix network and admixture analyses (Figures 2(d) and 3).

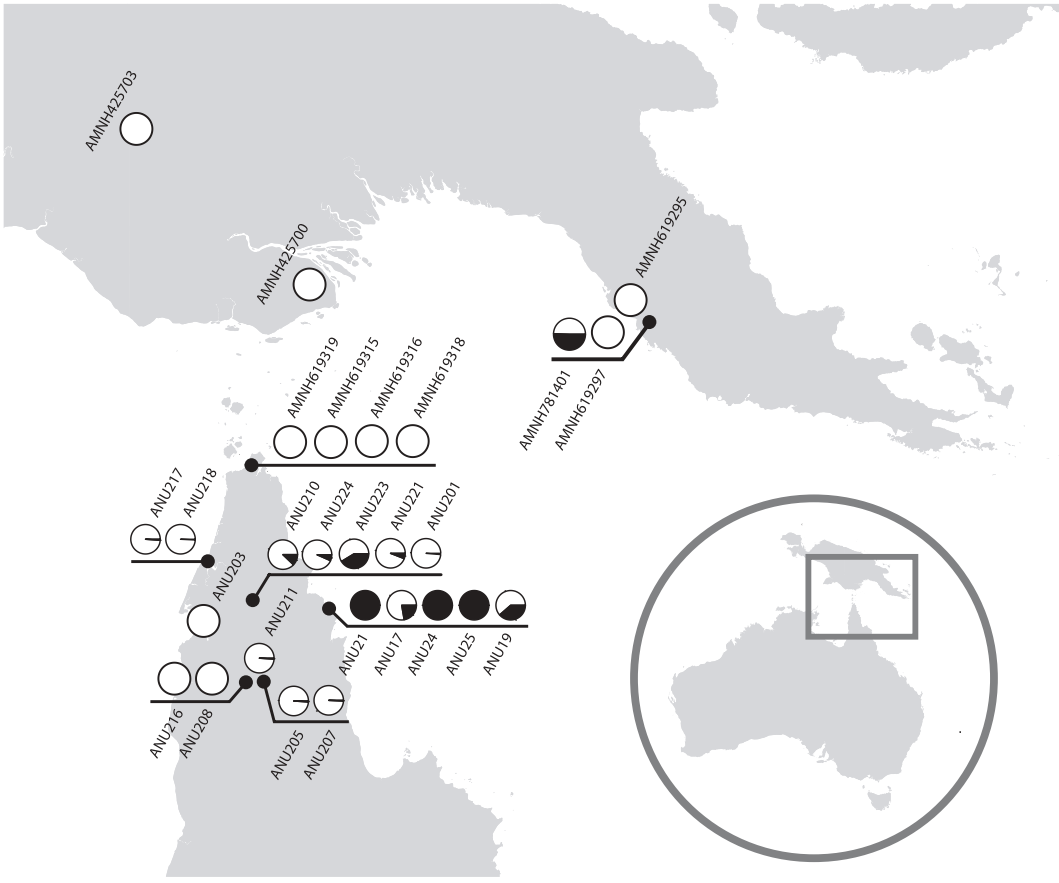


Figure 3. Admixture map. The map depicts the assortment of nuclear genomic SNP variation across individuals into two clusters (K = 2) with admixture analysis. Differentiation can be seen between Iron Range and other individuals from Cape York Peninsula and Papua New Guinea (PNG), though there is one mixed individual at Aroa, PNG. Central Cape York Peninsula individuals are more admixed than others, and two Iron Range individuals also display admixture.

### Population genetics statistics

The $F_{ST}$ estimate between the Iron Range (IR) population and the combined Cape York Peninsula (CYP) and Papua New Guinea (PNG) population was 0.514, which is characteristic of significant differentiation (Hartl and Clark 1997; Frankham *et al.* 2002). The absolute divergence measure is $D_{xy}$ = 4.98e-3 per site, with an equivalent relative divergence measure of $D_A$ = 4.97e-3 per site. This level of divergence is relatively high within subspecies of birds, but about average between subspecies (e.g. meliphagoid passerines; Peñalba *et al.* 2017). The per site heterozygosity (Watterson's θ) within each population is $\theta_{IR}$ = 5.42e-6 and $\theta_{CYP\&PNG}$ = 5.19e-6. The per site nucleotide diversity (π) within each population is $\pi_{IR}$ = 6.38e-6 and $\pi_{CYP\&PNG}$ = 8.39e-6.

### Species distribution modelling

The average AUC value for the MAXENT model was 0.87. After correcting for SSB, the cAUC resulted in 0.65. The average AUC value for the BIOCLIM model was 0.75. After correcting for SSB, the cAUC resulted in 0.58. The initial AUC values of both models satisfied the 0.7 threshold for predicting presence, and calibrated values (cAUC) for both models satisfied did not drop below 0.5, signifying greater predictive power than a null model (Hijmans 2012).

For all distribution projections, the climate-envelope BIOCLIM model had more restricted predictions than MAXENT (Figure 4). The mid-Holocene distribution projections of both models are more restricted than the present yet both methods show the Iron Range as suitable habitat at this time, which is the only area of suitable habitat on Cape York Peninsula according to the BIOCLIM model. Both modelled predictions for the last glacial maximum show that Palm Cockatoo habitat on Cape York Peninsula was even more restricted than in the mid-Holocene, with no suitable habitat predicted on Cape York Peninsula predicted by BIOCLIM. While the MAXENT prediction has a great deal of suitable habitat on an expansive land

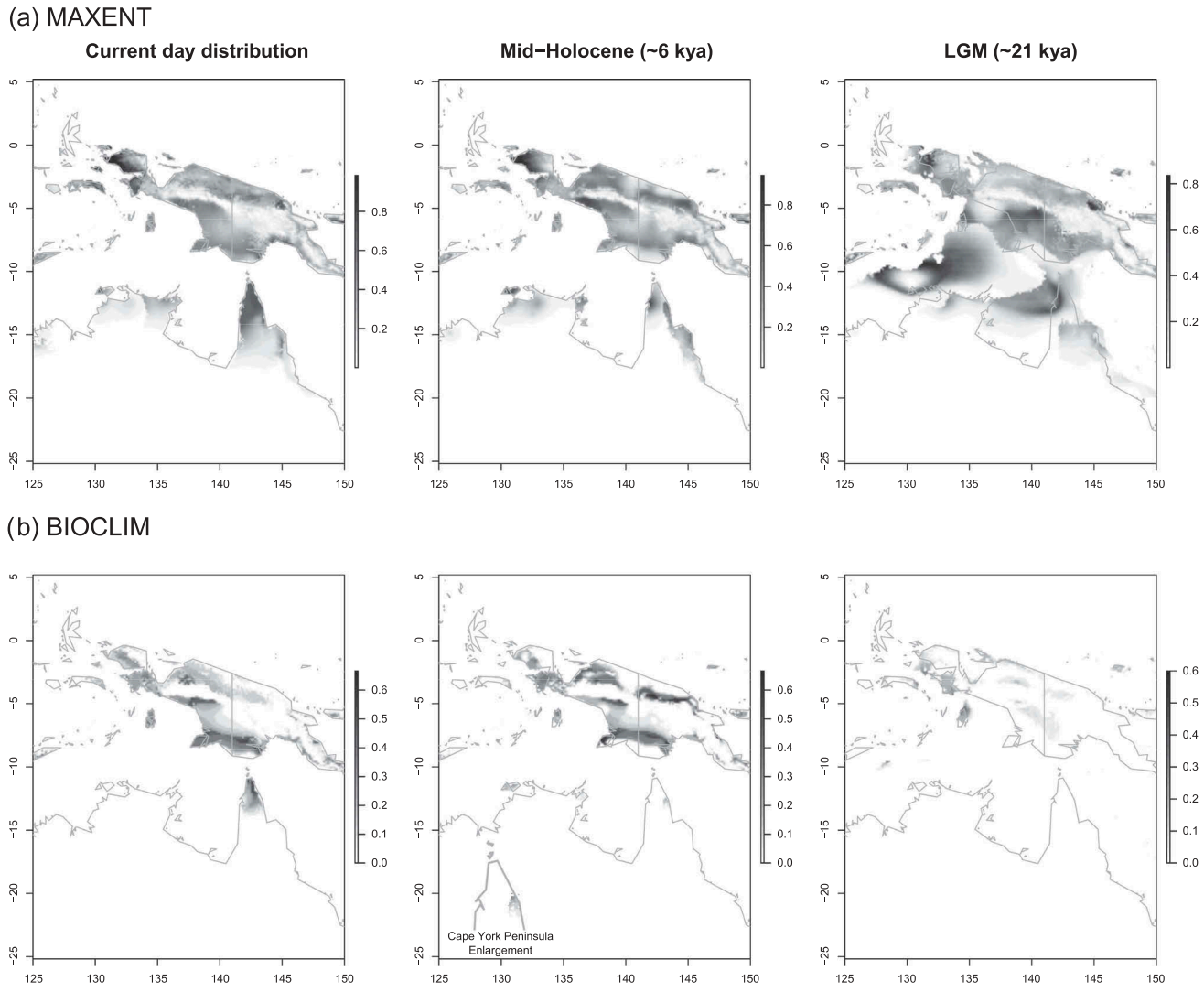


**Figure 4.** Species distribution modelling. Each map shows the predicted suitable range for the Palm Cockatoo species in Australia and Papua New Guinea, with shading indicating the percentage likelihood of occupation. (a) The top row corresponds to suitability predictions from a maximum entropy (MAXENT) model while the bottom row (b) corresponds to suitability predictions from a climate-envelope BIOCLIM model. The left column represents predictions for current-day habitat suitability with the published southernmost range extent on Cape York Peninsula indicated by a black line (Marchant and Higgins 1999), the middle column projects the predicted model to the climate of the mid-Holocene (~6 kya), and the third column projects the predicted model to the last glacial maximum (~21 kya).

bridge at the last glacial maximum, there is a gap in suitable habitat between Papua New Guinea and the Australian landmass.

## Discussion

Our results demonstrate the importance of examining population connectivity both in the context of historical biogeographic processes and from the viewpoint of modern population biology and cultural processes. We found a nuclear genomic structure within Cape York Peninsula that separated Iron Range Palm Cockatoos from other Australian populations, and which corresponded with a vocal dialect boundary. Admixture in SNP data provides preliminary evidence for some gene flow between Iron Range and other locations further west on Cape York Peninsula. We found a two bp difference in mitochondrial haplotypes between New Guinean and Australian birds, and a similarly divergent haplotype in one individual from the Iron Range. Our reconstructions of Palm Cockatoo past and present distribution had differing results depending on the inference method (BIOCLIM or MAXENT), though both suggest Palm Cockatoo

habitat on Cape York Peninsula has progressively expanded in distribution since the last glacial maximum. Below we explore in detail patterns of Palm Cockatoo persistence and recolonisation with regards to changing climate, effects on population connectivity, and the likely role of these processes in determining vocal characteristics.

### Genetic similarity between Cape York Peninsula and Papua New Guinea

Our data on nuclear SNPs show similarity between Cape York Peninsula Palm Cockatoos from outside the Iron Range and individuals from Papua New Guinea. This is consistent with sharing of haplotypes in the mitochondrial control region found by Murphy *et al.* (2007). Lack of differentiation either side of the Torres Strait supports either recent recolonisation of Cape York Peninsula from New Guinea following local extinctions or ongoing connectivity between extant populations until loss of the Torresian land bridge.

Our more restricted distribution reconstruction (BIOCLIM) suggests the absence of Palm Cockatoos on Cape York Peninsula at the last glacial maximum, with only small areas available by the mid-Holocene. Replenishment of Australian rainforest flora and fauna has been suggested via a connection during a short period ~7 kya when a warm-wet climate allowed expansive rainforest all along the Torresian land bridge which was not yet submerged (Nix and Kalma 1972). However, even during serious aridity, rainforest may have remained as small patches adhering to drainage routes or topographic features on Cape York Peninsula and the Torresian land bridge (Nix and Kalma 1972), which would make complete local extinction and recolonisation of Palm Cockatoos unlikely (Murphy *et al.* 2007).

Our more expansive distribution reconstruction (MAXENT) may allow connection of Australia's Palm Cockatoo habitat to New Guinea's at the last glacial maximum, but it also supports continual connectivity provided a land bridge persisted into the early/mid-Holocene. Savannahs and/or woodland are likely to have been present on the land bridge at this time, which is supported by close relationships between New Guinean and Australian Butcherbirds and Magpies (*Cracticus* spp.) (Kearns *et al.* 2014; Toon *et al.* 2017). Land bridge rainforest patches are supported by subspecies-level divergence among lower montane rainforest-reliant Catbirds (*Ailuroedus melanotis*) (Irestedt *et al.* 2016), Australian and New Guinean Bandicoots (*Echymipera rufescens*) (Westerman *et al.* 2001), and Pademelons (*Thylogale stigmatica*) (Macqueen *et al.* 2010). Species-level divergence in logrunners (*Orthonyx* spp.) that occupy higher altitude montane forest suggests a more ancient connection of this habitat type (Joseph *et al.* 2001). Lack of divergence in rainforest-dependent Red-bellied Pittas (*Erythropitta macklotii*) across the Torres Strait (Irestedt *et al.* 2013) can most likely be explained by their migratory life history. Non-migratory Sooty Owls (*Tyto tenebricosa*), which occupy both rainforest and woodland, lack significant genetic structure between Australian and New Guinean populations (Norman *et al.* 2002), which supports the existence of rainforest patches on the land bridge.

The persistence of Palm Cockatoos on Cape York Peninsula, rather than a recent complete replenishment, is weakly supported by our results with the fast-evolving mitochondrial *ND2* region. These results show some very low divergence, a two bp difference, and no haplotype sharing between New Guinea and Cape York Peninsula populations outside of Iron Range. We cannot completely distinguish between prolonged occupation of western Cape York Peninsula or a recent colonisation scenario based on our *ND2* results because of a possible founder effect (Provine 2004). Brief replenishment may have resulted in only one haplotype carrying over to Cape York Peninsula from New Guinea. Sequencing the *ND2* region of a greater number of New Guinea individuals is a suitable next step for confirming whether the Cape York haplotype evolved *in situ* with persistence, or is a result of a founder effect with replenishment.

### Genetic distinctiveness of the Iron Range

We found structure in nuclear SNP genomic data and a slightly divergent mitochondrial *ND2* haplotype in Palm Cockatoos from the Iron Range. Population differentiation as measured by $F_{ST}$ was much greater than mitochondrial and microsatellite differentiation among populations that display vocal dialects in other parrots (e.g. Yellow-naped Amazons (*Amazona auropalliata*); Wright and Wilkinson 2001; Wright *et al.* 2005). The unique mitochondrial *ND2* haplotype at Iron Range is consistent with unique mitochondrial control region haplotypes found there by Murphy *et al.* (2007). Evolution of additional haplotypes in conjunction with the results of historical distribution models supports occupation of Palm Cockatoos at Iron Range that was potentially longer in duration than elsewhere on Cape York Peninsula.

Both distribution models show restricted Palm Cockatoo distribution on Cape York Peninsula in the mid-Holocene and last glacial maximum, reflecting reduced areas with suitable climate for their habitat

(i.e. woodland with patches of rainforest). The BIOCLIM predictions were more severe with only low probability of suitable habitat in the Iron Range area in the mid-Holocene, with no suitable area at all on the Peninsula during the last glacial maximum. However rainforest pockets were probably preserved at the very least in topographic refugia (e.g. the escarpment of the Great Dividing Range) due to reliable orographic rainfall, which is better reflected in our MAXENT predictions. Enough rainforest for Palm Cockatoos was probably maintained at the Iron and McIlwraith Ranges given the persistence of other large rainforest-dependent vertebrates that have disappeared from rainforests elsewhere in Australia (Eclectus Parrots (*Eclectus roratus*); Legge *et al.* 2004; Green Pythons (*Morelia viridis*); Wilson and Heinsohn 2007). Evolution of a unique vocal dialect at Iron Range may have occurred in isolation in the refugial population there, similar to isolation-recombination dynamics creating dialect boundaries in other Australian parrots (e.g. Ring-necked Parrot (*Barnardius zonarius*); Baker 2008; Crimson Rosella (*Platycercus elegans*); Ribot *et al.* 2009, 2012).

We consider it unlikely that Iron Range samples cluster separately as an artefact of differential DNA degradation. Although the Iron Range samples were collected within the last 18 years and were of better quality than skin and feather tissue from specimens up to 102 years beforehand, the maximum sampling gap among specimens from within Cape York Peninsula is also 102 years and there is no concomitant differentiation. It is also unlikely that our structure reflects drift as a result of sampling different generations; firstly because Palm Cockatoos breed exceedingly slowly (about one successful offspring every 10 years; Murphy *et al.* 2003), and secondly because we again see no such generation gap among Cape York Peninsula samples.

### Contemporary population connectivity

Australian and New Guinean Palm Cockatoos represent separate management units due to their reluctance to cross the Torres Strait (Murphy *et al.* 2007). The separate clustering of nuclear loci for Palm Cockatoos from Iron Range on one hand and elsewhere on Cape York Peninsula on the other suggests the merit of managing them separately.

The northern section of the Great Dividing Range may separate the Iron Range population from the other areas on Cape York Peninsula. Palm Cockatoos are closely associated with the gallery-forest of river corridors (Murphy *et al.* 2003) and association with catchments on opposite sides of the Range may maintain population differentiation. However, we observed admixture between the two populations in some individuals at the Iron Range and along the Wenlock River in central Cape York Peninsula. The small distance (3 km) between the Wenlock (west-flowing) and Pascoe (east-flowing) Rivers at one point north of Iron Range may allow sufficient dispersal for the admixture we found. These results suggest that Wenlock River is a particularly important corridor between the two otherwise differentiated populations.

The Great Dividing Range (824 m at McIlwraith Range) itself is also a plausible barrier to dispersal as mountains explain population structure in other large parrot species (e.g. Scarlet Macaws (*Ara macao*); Olah *et al.* 2016), and Palm Cockatoos occur most commonly below 750 m in New Guinea (see Murphy *et al.* 2007 and references therein). A modelling approach based on hypotheses about contemporary connectivity may provide the extra information required for assessments of future population viability (Keighley *et al.* manuscript in preparation).

Despite differentiation representing separate populations, the divergence we found in nuclear SNPs was low compared to species, and subspecific-level structure in rainforest restricted birds of the region (e.g. logrunners; Joseph *et al.* 2001; Black Butcherbirds (*Cracticus quoyi*); Kearns *et al.* 2013; Catbirds; Irestedt *et al.* 2016). Low heterozygosity ($\theta$) and nucleotide diversity ($\pi$) within populations reflect either small effective population size or gene flow. Although an estimate of heterozygosity can be derived, parameter estimates of effective population size are highly sensitive to variation in how the ddRAD loci were filtered (Shafer *et al.* 2017). To remain conservative, we did not estimate the effective population size from these data. The results of our admixture analysis provide evidence that some gene flow may have ameliorated divergence of the Iron Range population. Although admixture signals from some samples may be an artefact of data completeness, a few better quality samples still provide preliminary evidence for gene flow. Increasing sampling would help better characterise the extent of gene flow and admixture between the Iron Range and surrounding populations. Some gene flow combined with the extremely slow life history strategy of Palm Cockatoos (Murphy *et al.* 2003) and the recency of the population split could explain the shallow divergence. The prevalence of the Cape York Peninsula *ND2* haplotype at Iron Range suggests more recent introgression than between Cape York Peninsula and New Guinea, which do not share haplotypes in this fast-evolving region of the genome.

There is a small possibility that emigration from Iron Range is less prominent since we do not find the unique *ND2* (or *CO2*; Murphy *et al.* 2007) haplotype from Iron Range elsewhere despite greater sampling effort representing the broader Cape York Peninsula population. As mitochondrial DNA is transmitted by females (Marais 2007), it is a speculative possibility that females are not leaving the Iron Range area. Furthermore, the slope of the Great Dividing Range escarpment is gentler from west to east (Figure 1(a)). This creates another speculative, though interesting, possibility that one-way dispersal into Iron Range could result from topographical gradients in a similar way to small-scale weather dynamics influencing sea bird movements (Schneider 1991). We consider it more likely, though, that any bias to dispersal into Iron Range would result from a disproportionately slow reproductive rate creating a population 'sink' there (Heinsohn *et al.* 2009).

### *Culture and connectivity*

Connectivity restrictions may also result from behavioural differences. Parrot vocalisations have social functions (Bradbury and Balsby 2016) and cross-dialect dispersers might experience greater difficulty establishing a territory, acquiring a mate or admission into social groups (Marler and Tamura 1962; Nottebohm 1969; Slabbekoorn and Smith 2002). Vocalisations and drumming behaviour feature significantly in Palm Cockatoo courtship displays (Zdenek *et al.* 2015) and drumming may be sexually selected (Heinsohn *et al.* 2017). Iron Range Palm Cockatoos have unique vocal dialects (Keighley *et al.* 2016) and may drum more frequently (pers. obs.). Immigrating males that drum less and are vocally different might have lower reproductive fitness and impeded social interaction (e.g. Yellow-naped Amazon Parrots; Salinas-Melgoza and Wright 2012) which might be maintaining the genetic divergence we find in the Iron Range population (see also Irwin 2000; Ribot *et al.* 2012).

Alternatively, vocal learning can preserve dialects without impeding gene flow (e.g. Wright *et al.* 2005; Baker 2008; Salinas-Melgoza and Wright 2012). However, dialect preservation can also reflect processes that limit cross-population movement such as short-distance dispersal and high philopatry (e.g. Yellow-naped Amazons; Salinas-Melgoza and Wright 2012). High philopatry is a known characteristic of Palm Cockatoos (Murphy *et al.* 2003) and could contribute to maintenance of their vocal dialects through limited dispersal.

## Conclusion

Our data provide evidence for considerable connectivity between resident Australian and New Guinean Palm Cockatoo populations prior to geographic separation by the formation of the Torres Strait. We also found genetic differentiation within Australian populations that aligns with vocal dialect boundaries. We show that genetic differentiation within Australia could have evolved in historical refugia, and probably coincided with the evolution of a unique vocal dialect there. Genetic mixing among Australian populations suggests some connectivity among vocally distinct populations, and that the distinct dialects are maintained through learning by immigrant individuals (as in Wright *et al.* 2005; Baker 2008). Our data also suggest the possibility that Palm Cockatoo dispersal into Iron Range outweighs dispersal out of the region, which alongside local declines characterise it as a 'sink' population (Heinsohn *et al.* 2009). Our results represent the only genetic study of Palm Cockatoos at the population scale. Although divergence is shallow compared to the subspecies-level structure in other species, preservation of unique vocal dialects, tool-use behaviour and the little remaining genetic diversity between the distinct subpopulations is a primary concern for future conservation management in this species. The unique characteristics of the Iron Range population make it key for future conservation effort.

## Acknowledgements

We would like to thank Sam Banks, Leo Joseph and Kerensa McElroy for advice and direction, George Olah and Todd Cross for advice on feather examples, Sonal Singhal for advice on using the Rainbow assembler and Matteo Fumagalli for assistance with ngsTools. For valuable assistance and companionship in the field we thank Chris Sanderson, Maddie Castles, Zoe Reynolds, Claudia Benham, Richie Southerton and Andrew Neilen. We thank Dejan Stojanovic for assistance with grant applications, Xenia Weber and two anonymous reviewers for feedback on the manuscript, and Julie Brokenbrow, Eridani Mulder and Kristy Phillikirk for providing feathers.

## Author contributions

The idea for this study was conceived by R.H. who, with N.E. L., contributed substantial resources, funding and edits of the manuscript. The molecular and modelling methods were developed, conducted and written by J.V.P., together with the results. The remaining manuscript was written with genetic samples collected and collated by M.V.K. S.A.M. extracted additional DNA from blood and tissue and commented on the manuscript.

## Ethical note

This research was conducted under an Australian National University ethics protocol (No. A2012/36).

## Funding

This research is supported by an Australian Government Research Training Program Scholarship. We would like to thank the Hermon Slade Foundation and the National Geographic Society for funding fieldwork, and BirdLife Australia for funding lab costs through the Stuart Leslie Bird Research Award.

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