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Identifying conservation units after large-scale landclearing: a spatio-temporal molecular survey of endangered white-tailed black-cockatoos (*Calyptorhynchus* spp.)

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ABSTRACT

Aim We examined how the threatened and endemic white-tailed black-cockatoos of Western Australia have responded genetically to recent and comprehensive habitat loss with the ultimate aim of identifying units for conservation. We assessed the population structure, connectivity and genetic diversity at spatial and temporal scales for *Calyptorhynchus baudinii* and *C. latirostris*, which have undergone dramatic population declines. Genetic comparisons of pre- and post-population decline were carried out by including historical samples dating back to 1920. We examined samples collected from across 700 km of their distribution and sampled ~1% of the current population census size to produce significant insights on the population genetics of white-tailed black-cockatoos and generate genetic information crucial for conservation management.

Location Southwest corner of Western Australia.

Methods Six hundred and eighty-four cockatoo samples were collected from 1920 to 2010 and profiled with 19 microsatellites to identify spatial population structure and loss of genetic diversity.

Results The temporal and spatial microsatellite data illustrated that the geographically defined genetic structuring in white-tailed black-cockatoos is likely to represent a recent phenomenon. We identified; (i) spatial population substructure east and west of extensively cleared habitat (>95,800 km^2), but the historical samples clustered with the current western population, regardless of origin; (ii) a regional loss of allelic diversity over 3-4 generations for the current eastern population; (iii) a lack of a genetic signal of the recent population decline, but perhaps a mid-Holocene population collapse; and lastly, (iv) limited genetic differentiation between the two currently recognised white-tailed black-cockatoo species suggests a review of taxonomy and/or management units should be undertaken.

Main conclusion Based on extensive spatio-temporal sampling, we have demonstrated that recent anthropogenic habitat modifications have affected the genetic structure of a long-lived

and highly mobile species. Our results have identified areas of high conservation value and the importance of maintaining native vegetation migration corridors.

Keywords: *Calyptorhynchus*, cockatoos, microsatellites, population structure, ancient DNA, historical sampling, habitat fragmentation, southwest Australia

Introduction

It is widely acknowledged that anthropogenic habitat destruction and fragmentation are major causes of declines and extinctions of terrestrial biota (Harrison & Bruna, 1999; Fischer, 2000; Pimm & Raven, 2000). From an animal's perspective, habitat loss and fragmentation can lead to discontinuities in the distribution of resources such as food, water and shelter, and a reduction in connectivity among sub-populations (Segelbacher *et al.,* 2010). Moreover, isolated sub-populations become the units on which genetic drift act (Young & Clarke, 2000; Frankham *et al.,* 2002; Toro & Caballero, 2005; DiBattista, 2008), potentially leading to rapid erosion of genetic diversity, which can cause a reduction in fitness, restrictions on evolutionary potential and increase the extinction risk (Frankham, 1998, 2009; Frankham *et al.,* 2002; Spielman *et al.,* 2004; Evans & Sheldon, 2008). For this reason the World Conservation Union (IUCN, 2010) recognizes the preservation of genetic diversity as a global conservation priority (McNeely *et al.,* 1990) and maintaining functional connectivity of populations is an important aim of conservation. This is particularly important for areas containing high biodiversity.

Avifauna worldwide, have declined in range and abundance with cascading impacts on biodiversity (Dudaniec *et al*., 2011; Sunnucks, 2011). Birds often play a central role in food chains; being both predators and prey, pollinators and seed dispersers, and are therefore important bio-indicators of overall ecosystem health (Anderson *et al.,* 2011). In reviewing 194 bird studies, Evans & Sheldon (2008) examined correlates of genetic diversity with respect to conservation status, population size and extinction risk. It is not straightforward to demonstrate causality (i.e., is a population decline causing loss of genetic diversity, or is lack of genetic diversity leading to population decline?) but regardless, their study concluded that bird species at risk of extinction often display relatively low levels of genetic diversity. Such

results provide an impetus to examine the spatial and temporal effects of habitat fragmentation on the gene pools of birds. As many species of bird have the ability to disperse widely, excepting ground dwelling species (e.g. Caizergues *et al.,* 2003), they may be less prone to loss of genetic diversity, as gene flow among sub-populations can perhaps be maintained across large distances of poor habitat (Bates, 2000; Galbusera *et al.,* 2004).

In southwestern Australia, around 90% of the original vegetation has been cleared for agriculture since Europeans arrived (Saunders *et al.,* 1985; Saunders, 1989; Williams *et al.,* 2001). The Southwest Australia Ecoregion (SAE) is one of 34 internationally recognised global biodiversity hotspots, owing in part, to species richness and a high level of endemism (Myers *et al.,* 2000). The Avon Wheatbelt (AW) is centrally located in this ecoregion (Fig. 1). It is an area of 95,800 km^2 (equivalent to the size of Portugal or Hungary) of broad-scale agriculture. The AW comprises over 20% of the total land area in SAE, and has 7% of native vegetation remaining (Williams *et al.,* 2001). As a consequence, 50-60% of the Wheatbelt avifauna has declined in range and/or abundance (Saunders & Ingram, 1995; Olsen, 2005). Based on the distribution of endemic white-tailed black-cockatoos and the extent of land clearing (Fig. 1), we hypothesise that the AW effectively constitutes a giant 'wedge' of unfavourable habitat that acts as a barrier to gene flow.

White-tailed black-cockatoos (Genus *Calyptorhynchus*) are large (~650g) long-lived, monogamous species with an estimated generation time of 15 years (Saunders 1982; Saunders & Dawson, 2009). Sexual maturity is reached at four years of age and one to two eggs are laid per annum. It is estimated that 14.8% of juvenile birds survive their first year (Saunders, 1982). The following factors such as; (1) a slow rate of reproduction; (2) their longevity; (3) their period of immaturity and juvenile survival; (4) their loyalty to breeding and foraging areas; (5) the long association between parents and offspring; and (6) the strong bond between

a mating pair – are all advantageous in a stable environment, but place the species at a disadvantage during periods of rapid environmental change. Two species are currently recognised, *Calyptorhynchus baudinii* (long-billed white-tailed black-cockatoo or Baudin's Cockatoo) and *C. latirostris* (short-billed white-tailed black-cockatoo or Carnaby's Cockatoo). Hereafter, both species of white-tailed black-cockatoo will be referred to as WTBCs.

It has been estimated that WTBCs have suffered range contraction and reductions in population size (Saunders, 1990; Saunders & Ingram, 1998). Throughout their range suitable food and breeding resources are becoming increasingly rare and this is compounded with increased competition with other bird species (Saunders, 1979; Saunders *et al.,* 2003; Burnham *et al.,* 2010). Additionally, these cockatoos are illegally poached and traded for the pet market, and sometimes illegally killed for exploiting resources on fruit and nut orchards (White *et al.,* 2012). They are internationally listed as endangered by the International Union for Conservation of Nature (IUCN Red List v3.1). WTBCs are currently being threatened on several fronts and there is an urgency to understand their population structure and connectivity to develop efficient management strategies.

We sampled 660 birds (adults, sub-adults and nestlings) extending across a 700 km range to investigate the spatial population genetic structure of WTBCs. We included 24 historical samples from museum specimens to compare the genetic structure pre- and post-population decline and assess the temporal genetic impacts of habitat loss and fragmentation. We specifically aimed to examine how the species have responded genetically to recent and comprehensive habitat loss. In addition to yielding general insights on the conservation genetics of WTBCs, this assessment should generate novel genetic information that can be recognised in conservation planning and management strategies.

Material and Methods

Sampling

A total of 684 white-tailed black-cockatoo samples were collected from the SAE (Fig. 2). Samples of blood, feather, and/or tissue were collected between 1920 and 2010. The samples were categorised into species and various life stage groups as 'nestlings' (*n* = 347; *C. latirostris*), sub-adults/adults (*n* = 313; 73 *C. baudinii* and 240 *C. latirostris*), which were of known provenance (Table S1), but unknown place of birth (Mawson, 1997; Berry & Owen, 2009; Cale, 2003; Saunders *et al.,* 2011). Nestlings were sampled during the latter part of the breeding season (October to January). Sampling from nest hollows constituted considerable logistical challenges, but the data were important to obtain in order to assess if breeding ground fidelity was affecting the overall genetic structure. In addition to the 660 contemporary individuals (Table S1), historical specimens (*n* = 24; 9 *C. baudinii* and 15 *C. latirostris*) were included (Table S2). Further details are provided in the supporting information.

DNA extraction and microsatellite genotyping

Tissue and feather samples were preserved in 20% DMSO, blood samples were frozen in EDTA and historical feathers were stored in a clean, dry eppendorf tube. DNA extraction technique varied depending on the origin of the sample. In general, this was either using a salting-out technique (Miller *et al.,* 1988) or using Chelex® resin (Walsh *et al.,* 1991). For the historical samples, DNA was extracted with QIAamp DNA Micro kits (Qiagen), following a modified protocol from Bush (2007). See supporting information.

Genetic profiles were generated from 19 microsatellite markers detailed in White *et al.,*

(2009). Genotypic data were examined for allelic dropout and null-alleles within the program ML-Relate (Kalinowski *et al.,* 2006; Kalinowski & Taper, 2006), and the data were examined for identical multi-locus genotypes in GenAlEx v6.41 (Peakall & Smouse, 2006).

Genetic diversity and uniqueness

Allelic frequencies and diversity measures; number of alleles (N_A), the mean expected (H_E), and observed (H_O) heterozygosities, and the number of private alleles (*Pa*) were estimated in GenAlEx. A rarefaction method (Petit *et al.,* 1998) was used to assess allelic (*Ar*), and private allelic (*PAr*) richness, which was calculated using HPrare (Cornuet & Luikart, 1996; Petit *et al.,* 1998; Kalinowski, 2004, 2005) with a non-parametric Wilcoxon signed-rank test to test the significance in SPSS v15. Deviations from Hardy-Weinberg proportions and linkage disequilibrium were tested in GENEPOP v4.0.10 (Raymond & Rousset, 1995a).

Genetic structure, differentiation and gene flow

We used STRUCTURE v3.2.1 (Pritchard *et al.,* 2000; Manel *et al.,* 2005; Hubisz *et al.,* 2009) to investigate the genetic structuring. See supporting information. The most likely number of genetic units (*K)* was calculated with the method by Evanno *et al.,* (2005) using StructureHarvester v0.6.1 (Earl, 2011). We compared corrected estimates of the admixture proportions (*Q*) using a likelihood ratio test, and evaluated the probability of assignment to each genetic unit and cluster. Individuals were assigned to a genetic unit when *Q* was ≥ 0.75.

A spatial Bayesian clustering analysis using the program TESS v2.3.1 (Chen *et al*., 2007) was used to investigate the population substructure of nestlings (n = 347), with individual birthplaces as the standard input (Durand *et al*., 2009). TESS seeks population structure from individual multilocus genotype samples at distinct geographical locations without assuming predefined populations. The program builds individual neighbourhood networks using the

Voronoi tessellation. The Deviance Information Criterion was used to estimate the number of genetic units.

We quantified genetic differentiation with F_{ST} values within the AMOVA framework (Excoffier *et al.,* 1992) in GenAlEx v6.4, and used 999 permutations to test the significance. We also employed a direct test for allele frequency differences using the probability test (exact G test) (Raymond & Rousset, 1995a) in GENEPOP v4.0.10 (Raymond & Rousset, 1995b). The private alleles method of Slatkin (1985) was used as an indirect estimator of number of migrants per generation (*Nm*).

A Mantel test in the program IBD web service v3.23 (Jensen *et al*., 2005) was used to test for Isolation-by-distance. The correlation between the genetic distance (pair-wise $F_{ST}/(1-F_{ST})$) values and logarithm) and geographic distance (kilometres and logarithm) were assessed by 10,000 permutations with the slope and intercept of the IBD relationship calculated with a reduced major axis regression.

Detection of demographic change

An estimate of effective population size (N_e) based on linkage disequilibrium was carried out using LDNE (Waples & Do, 2008). This approach accommodates data from a single time point as opposed to more commonly used temporal methods (Luikart *et al.,* 2010). Although historical data were available, the temporal method was not seen as appropriate to apply due to the considerable distances in time and space between historical sampling points (Fig. 2; Table S2).

We investigated the occurrence of demographic population changes using BOTTLENECK (Piry *et al.,* 1999), and MSVAR (Beaumont, 1999; Storz & Beaumont, 2002). Detailed

methodology is provided in the appendix (Tables S3 & S4). For MSVAR the parameters of interest were: current effective population size (N_0), ancestral effective population size at the time of demographic change (N_1), and time (T) since the population started changing in size (Girod *et al.,* 2011). Severe demographic changes are inferred when $N_0/N_1 \leq 0.01$ (contractions) or $N_0/N_1 \geq 100$ (expansions). Due to computational intensity and the fact that analyses such as this for inferring demographic history should consider the violations of the panmixia assumption (Heller *et al*., 2013), MSVAR analyses were restricted to 100 random nestling samples from the western region using 10 microsatellite loci.

Results

Locus characteristics and Genetic diversity

Significant deviations from Hardy-Weinberg (HW) proportions (Bonferroni corrected α of 0.05) were observed in 13 of 19 loci for the contemporary dataset and one locus for the historical dataset. We found no evidence for linkage disequilibrium and all 19 loci were therefore retained for analyses. All microsatellite loci were variable and measures of genetic diversity are shown in Table 1. Separation of the data into species groups showed that they had similar levels of H_E and allelic richness (Table 1).

The historical dataset did not markedly differ from the contemporary sample group in terms of genetic variation, as reflected in both expected (H_E = 0.70 and 0.72) and observed (H_O = 0.59 and 0.64) heterozygosities (Table 1 & S6). The historical dataset was found to contain a single allele that was absent from the contemporary dataset.

Of 684 samples, 683 showed unique multi-locus genotypes. We identified a nestling as a 100% match with a bird found dead, west of Esperance in January 2010 (Fig. 2).

Identification from the leg band confirmed this genetic match. It was 103 km from its natal area , 13 months after it had been banded (Saunders *et al.,* 2011).

Pair-wise measures of genetic differentiation for the species groups were small (<0.05) (Table S7). Despite low to moderate genetic differentiation, the exact tests, showed ($P < 0.001$) that the allele frequencies for the species groups could not have been drawn from the same underlying distribution. The overall magnitude of migration, based on the private alleles method, was found to be high, with an average estimate of migrants per generation of Nm = 10.51.This result mirrors the low to moderate F_{ST} values, as estimated above, and although unlikely, we cannot completely exclude the possibility of unsampled populations, therefore the exact value of Nm should be interpreted with caution.

Population substructure

When analysing the contemporary and historical data together (n = 684) in STRUCTURE, each of the 20 independent analyses yielded two (K=2) genetic units (Fig. 2). Twenty-two of the 24 historical samples (92%) and 614 of the 660 contemporary samples (93%) assigned with high admixture proportions ($Q \geq 0.75$) to one of the two genetic clusters identified (Fig. 2 & 3). The corrected posterior probability for K=2 was significantly higher than K=1 (likelihood ratio = 1019.2, df = 1, $P < 0.001$). Results for K=2 revealed that the partition was defined by two well-differentiated clusters, roughly corresponding to the areas east and west of the AW from where the samples were collected (Fig. 2 & 3; Table S5).

When we analysed nestlings (n = 347) both STRUCTURE and TESS consistently identified two (K=2) genetic clusters. The same result was obtained for the sub-adult/adult (n = 313) dataset. When we tested for the presence of three genetic clusters (K=3), to examine the species groups, *C. latirostris* and *C. baudinii*, in the western region (Fig. 1 & 2), the third

cluster contained 95% of birds that had been morphologically identified as long-billed WTBC (Fig. 2 &3). However, the overall probability was significantly lower than for K=2 (likelihood ratio = 555.7, df = 1, $P < 0.001$), implying that the overall substructure in WTBCs seems to be defined by the AW (eastern and western populations), and that any further structuring of the gene pool (amongst *C. latirostris* and *C. baudinii*) is much weaker.

The Mantel test showed that there was no correlation between genetic and geographic distance (r = -0.037, P = 0.5), or logarithmic genetic and geographic distance (r = -0.124, P = 0.56) of the sample groups. Therefore the genetic structure of WTBCs in the SAE does not seem to be the result of an isolation-by-distance pattern according to the Mantel test (Fig. S1). An important objective was to test if the extensive clearing of native vegetation in the SAE (Fig. 1) has resulted in measurable genetic structuring in WTBCs. This hypothesis has now been confirmed and the remaining results are therefore discussed in relation to the east-west geographic structuring.

Genetic diversity and differentiation of STRUCTURE populations

Separation of the data into the populations as determined by STRUCTURE ($Q \geq 0.75$; n = 614) showed similar levels of H_E (0.69 and 0.72) and allelic richness (6.10 and 6.67) for the eastern and western populations, respectively (Table 1). However, in terms of the number of private alleles and richness, the western population contained eight times the number of private alleles (Table 1). A Wilcoxon signed-rank test confirmed the difference as significant (P = 0.028). The magnitude of migration showed an average Nm = 9.4 after correction for sample size. Measures of genetic differentiation indicated a low (F_{ST} = 0.028), but highly significant (P = 0.001) degree of differentiation. Under the definition by Lowe & Allendorf (2010), the populations are at 'drift genetic connectivity' (F_{ST} ~0.02), as there is sufficient gene flow to maintain similar, although not identical allele frequencies.

Effective population size, demographic change and bottleneck

The effective population size was estimated at 736 individuals (95% CI: 671 – 810) for the entire population, 746 (95% CI: 661 – 850) and 535 (95% CI: 445 – 660) individuals for the western and eastern regions, respectively. The overall *N*e to census size ratio (*N*e/*N*c), using the lowest and highest estimates (*N*c 40,000 – 75,000 birds; Saunders *et al.,* 1985; Burnham *et al.,* 2010), ranged from 0.010 to 0.018. We found no support for a recent genetic bottleneck despite knowledge of a dramatic decline in the demographic size over the past ~50 years. The Wilcoxon test revealed no significant heterozygosity excess ($P = 0.999$).

Although the results from MSVAR were accompanied by large confidence intervals, the results strongly supported a demographic decline scenario (Fig. 4). The estimate of current effective population size (median) was found to be $N_0 = 250$ (95% CI: 51 – 3,467), and an ancestral effective population size of $N_1 = 28{,}800$ (95% CI: 4571 – 165,958). Despite the wide confidence intervals in the temporal estimates, we note that the 95% CIs on N_0 and N_1 are not overlapping (Fig. 4). The timing of the decline (T) also carries considerable errors, but even considering this error (Fig. 4), it seems that MSVAR is detecting a more ancient, perhaps mid-Holocene, bottleneck in WTBCs, rather than the well-documented late 20th century decline: $T = 3{,}388$ years before present (95% CI: 91 – 83,176) (Fig. 4).

Discussion

Population structure of white-tailed black-cockatoos

An accurate identification of population structure is central to best-practice conservation, as populations often represent focal units for determining appropriate management (Moritz, 1994; Waples & Gaggiotti, 2006). In the case of the WTBCs, we hypothesised that the AW

constitutes a significant dispersal barrier, giving rise to population sub-structuring (Fig. 2 & 3). The AW is internationally recognised for the severity of loss of native vegetation, a long list of extirpations, and a fauna that is the most negatively affected in all of Western Australia (May, 2003). Prior to the extensive clearing (commencing in the 1820s but most intense between 1945 and 1982), it seems highly probable that WTBCs represented a single unit with no distinct population subdivisions. Data from our historical samples support this hypothesis, as 92% of them, regardless of where they were collected, clustered ($Q \geq 0.75$) with the contemporary samples from the western region (Fig. 2). The results from the historical dataset therefore suggest that the geographically defined genetic structuring in WTBCs is likely to represent a recent phenomenon. It is evident that the historic samples carried allele frequencies more similar to the present day western region birds. Although our data were not found to support a decline of genetic diversity (Table 1), we detected a change in allele frequencies amongst WTBCs of the eastern region (Fig. 3).

The current white-tailed black-cockatoo population is spatially structured and it seems likely that the massive 'wedge' of cleared land through the centre of *C. latirostris'* range has contributed to the observed genetic structure. Our examination showed no support for a correlation between genetic and geographic distance (Fig. S1) supporting our finding of recent genetic structuring. White-tailed black-cockatoos undertake seasonal migrations to and from their breeding grounds over distances of 100-250 km (Saunders 1980; Johnstone & Kirkby, 2008). Sub-adult females choose their mate during the periods when large flocks form outside of the breeding grounds and, at least with regard to *C. latirostris*, females have been documented to return to their natal area to breed (Saunders & Ingram, 1998). From the historical and isolation-by-distance data we know that the current genetic structure did not predate the removal of the bulk of native vegetation. Therefore, our results seem to suggest that the creation of the AW has reduced the opportunities for sub-adult females from either

the western or eastern regions to meet prospective mates from the opposing region during non-breeding aggregations. The spatial genetic differentiation between the eastern and western populations is not substantial (F_{ST} = 0.028), but nonetheless highly significant. This suggests that allele frequencies of WTBCs inhabiting the eastern and western regions have drifted apart over a relatively short time scale or, alternatively, that there has always been a genetic gradient from east to west, but clearing of native vegetation either eliminated or polarised the entire 'middle' of the spectrum (Fig. 1 & 2).

Since the historical specimens cluster with the current western birds, it seems that it is *C. latirostris* of the eastern region that have genetically 'drifted away' from the rest. This is further supported by the observation of only a few private alleles from this area. We have no data at present to explain why *C. latirostris* inhabiting the eastern region could be more genetically affected than the western birds, except for the obvious difference in the amount of remnant vegetation (Fig. 2) and possibly smaller population size. Only in the extreme southwest are there large intact jarrah, marri and karri forests (southern Jarrah Forest and Warren IBRA regions; Fig. 1). Recent population census size counts from 2012 recorded the largest number of WTBCs in these regions, in addition to the northern Swan Coastal Plain (Fig. 1; Kabat *et al*., 2012). When differences in resource quality among populations translate into differences in demographic rates then spatially structured population dynamics may result (Bonebrake & Beissinger, 2010; Lowe & Allendorf, 2010). It could be that the areas of remnant native vegetation of the western region are more attractive and able to support larger numbers of WTBCs than those of the eastern region.

The genetic connectivity of WTBCs populations was indirectly assessed from F_{ST} values and indications are that some gene flow is still occurring. However, less than 10 immigrants per generation are moving between the regions suggesting that the AW constitutes a significant

dispersal barrier. It is clear that the extensive changes to the landscape in Western Australia since European settlement have had a significant role in shaping the gene pools of WTBCs. A well-documented loss of breeding populations (Saunders, 1982, 1986, 1990) and decline in census numbers in recent decades represents the most compelling evidence that WTBCs have been severely affected by the extensive destruction of their habitat. This combined with stochastic weather events (Saunders *et al.,* 2011) is cause for concern for their long-term survival.

Genetic diversity in white-tailed black-cockatoos

Despite the extensive habitat loss and 50% decline in census size, the genetic diversity for WTBCs was not particularly low (Table 1). In addition, the contemporary and historical datasets indicate that WTBCs have lost little or no genetic diversity in the last century. This finding parallels others, such as capercaillie (Segelbacher *et al.,* 2003), white-tailed eagle (*Haliaeetus albicilla*; Hailer *et al.,* 2006), saddleback (*Philesturnus carunculatus*; Taylor & Jamieson 2008), Spanish imperial eagle (*Aquila adalberti*; Martinez-Cruz *et al.,* 2007), and British golden eagle (*Aquila chrysaetos*; Bourke *et al.,* 2010). Our findings seem to support Hailer *et al.,* (2006) who argued that the long generation time of eagles has acted as an intrinsic buffer against loss of genetic diversity, leading to a shorter effective time of the experienced bottleneck. Others have suggested that ancient bottlenecks can have a long-term effect on genetic variation, to such an extent that a more recent population decline may leave no genetic signature (Segelbacher *et al.,* 2003; Taylor & Jamieson, 2008). The apparent retention of genetic diversity in WTBCs is favourable for their long-term adaptability (Frankham, 2005) and this combined with current and future directed on-the-ground recovery efforts should provide WTBCs a chance for survival in the future.

Genetic bottleneck and effective population size

The lack of a signal of a recent genetic bottleneck suggests that the well-established population decline has not had a pronounced effect on the genetic diversity of WTBCs (Table 1). WTBCs do not breed until they are four years of age, they usually fledge only one young per year (Saunders, 1982), and are long-lived with records of birds surviving for more than 30 years in the wild (Saunders & Dawson, 2009). Therefore, the timing of the extensive habitat changes occurred only three to four generations in the past. Our MSVAR analysis detected an ancient bottleneck, estimated to have occurred prior to European settlement (early 1800s) and possibly during the mid-to-late Holocene (Fig. 4). White *et al.,* (2011) estimated white- and yellow-tailed black-cockatoos (*C. funereus*) evolved from a common ancestor approximately 1.3 million years ago, and the lack of DNA variation between *C. baudinii* and *C. latirostris* suggested that a significant decline had occurred during the last glacial maximum. This could be the bottleneck we detected with MSVAR. Alternatively, it is generally accepted that Aboriginal land-use practices (dating back c. 49,000 years in the region) had a significant impact on vegetation distribution (Hopper *et al.,* 1998; Gammage, 2011), which could have affected the survival of these birds in pre-European times.

The size of a population is negatively correlated with proneness to extinction, so estimates of population size are valuable in predicting persistence (Sunnucks, 2011). Reliable census size numbers can be logistically challenging to achieve for a volant species, but the total number of WTBCs has been estimated at 40,000 – 75,000 (Saunders *et al.,* 1985; Burnham *et al.,* 2010). Our genetic estimate (LDNE and MSVAR) of *Ne* ranged from a mean of 250 to 746 individuals with a *Ne*:*Nc* ranging from 0.3 to 1.9%, which is lower than expected (Frankham, 1995). There can be several reasons for this 'discrepancy'. Disregarding potential inaccuracies in field counts and genetic estimates, the number could reflect the biology of WTBCs, which parallels the finding of Russello *et al.,* (2010) for the Bahama parrot (*Amazona leucocephala*). However, our result could also suggest that breeding conditions or

recruitment and fledgling survival for WTBCs are presently poor, allowing only a small fraction of the adults to produce and raise offspring. The effective population size is inversely proportional to the rate at which genetic drift alters neutral genetic variability (Waples & Yokota, 2007; Palstra & Ruzzante, 2008; Luikart *et al.,* 2010), so this low *Ne* could explain why the allele frequencies between the eastern and western regions have drifted apart within only three to four generations.

Conservation implications

In Australia, broad-scale clearing of native vegetation has been concentrated in the southwestern, southern and northeastern coastal, and sub-coastal regions. Clearing has been extensive (~400,000 km^2) and is still ongoing (State of the Environment Advisory Council, 1996; Burgman & Lindenmayer, 1998; Sunnucks, 2011). The detection of spatial genetic subdivision, as a result of the extensive clearing, implies that both western and eastern regions are important for conservation of WTBCs. If the population in either region is extirpated, it is questionable whether a new population can establish itself by natural recruitment. As shown by Saunders (1982, 1986) local extirpations have been occurring over the last 30 years and recent field surveys indicate that these may continue. Importantly, our analyses have provided new insights into the genetic diversity of the eastern region, which has been poorly studied ecologically. We have identified that *C. latirostris* of the eastern region have undergone a loss of allelic diversity and have drifted genetically from the western population, which still resembles the historic gene pool.

Although the eastern birds are perhaps under the greatest immediate pressure, it is of interest and conservation significance that the WTBCs of the western region show high adaptive potential to local environments, demonstrated by the presence of two bill morphotypes. Our results and the finding of White *et al*., (2011) support a scenario of morphological and

ecological variation with little genetic divergence. White-tailed black-cockatoos comprise one evolutionary significant unit (ESU; Moritz, 1994) and the overall genetic structure in WTBCs is defined by landscape. Our data, in addition to White *et al.,* (2011), strongly suggests that the taxonomy of WTBCs should be reviewed.

The 2012 recovery plan for *C. latirostris* states it is recognisable morphologically, behaviourally and ecologically from *C. baudinii* (Department of Environment and Conservation, 2012). For these reasons and the species' genetics, three management units (MUs) could be assigned with the allocation of two MUs for *C. latirostris* populations (east and west) that addresses their current population structure (Moritz, 1994), and an additional MU for *C. baudinii.* Our argument may be that of a pragmatic one for conservation, however, the birds of the western region are genetically and morphologically differentiated from the eastern population and conservation management should acknowledge the importance of this region for ensuring both the genotypic and phenotypic diversity is maintained. It is clear that the western population maintains the genetic signatures of pre-anthropogenic decline and is an important source of genetic and morphological diversity for WTBCs. However, as land clearing continues for urban development and illegal shooting and poaching continues, so too will further declines. Therefore conservation management should prioritize and mitigate these threats.

Habitat loss is the single greatest threat to woodland birds in Australia and research has illustrated that loss of avian species diversity is greatly accelerated when less than 30% of native vegetation remains (Morgan, 2001; Williams *et al.,* 2001; Olsen, 2005; Sunnucks, 2011). Of paramount importance are the protection, enhancement and expansion of native vegetation corridors, specifically those of the southern Jarrah Forest. We hypothesize that the southern Jarrah Forest adjoining the Esperance Plains and Mallee regions (Fig. 2) is

facilitating a fragile demographic and genetic connectivity between the regions. It seems likely that this residual native vegetation corridor may be the only remaining dispersal route supporting gene flow between the genetically spatial sub-structured regions. The preservation of this corridor should be a major conservation goal for WTBCs, and also for a long list of unstudied organisms that are likely to be equally affected by the destruction of native vegetation.

Conclusions

This study has generated important new information as to how habitat fragmentation can rapidly affect a species gene pool. Although the recent genetic changes in WTBCs are subtle and have not resulted in obvious loss of alleles from the overall gene pool, we have still documented rapid genetic differentiation and a regional loss of genetic diversity. We also note that because the timing of the establishment of this major dispersal barrier is known, the AW offers an excellent opportunity to study micro-evolutionary implications of habitat destruction and gene flow barriers across a wide range of species. Being listed as an internationally recognised biodiversity hotspot such studies are highly relevant. Lastly, our study highlights the need to consider genetic processes operating at both a landscape scale and over time. We strongly advocate the use of both spatial and temporal data to understand post-fragmentation levels of vagility and genetic diversity.

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Supporting Information

Identifying conservation units after large-scale landclearing: a spatio-temporal molecular survey of endangered white-tailed black-cockatoos (*Calyptorhynchus* spp.)

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Material and Methods

Sampling

A total of 684 white-tailed black-cockatoo samples were collected from the Southwest Australia Ecoregion (Fig. 2). Samples of blood, feather, and/or tissue were collected between 1996 and 2010. The samples were categorised into species and various life stage groups as 'nestlings' (*n* = 347; *C. latirostris*) sampled directly from nest hollows (Table S1) or 'adults/sub-adults' (*n* = 313; 73 *C. baudinii* and 240 *C. latirostris*), which were of known provenance (Table S1), but unknown place of birth. Nestlings were sampled during the latter part of the breeding season (October to January). Sampling from nest hollows constituted considerable logistical challenges, but the data were important to obtain in order to assess if breeding ground fidelity was affecting the overall genetic structure. Nestlings were between three and 10 weeks old when feathers were collected. The 313 adults/sub-adults were collected from monitored roost-sites (Berry & Owen, 2009), from rehabilitated, injured, or deceased birds, and from private aviaries (Mawson, 1997; Cale, 2003; Saunders *et al.,* 2011) where the birds' location (in the wild) and date had been recorded. In addition to the 660 contemporary individuals (Table S1), historical specimens (*n* = 24; 9 *C. baudinii* and 15 *C. latirostris*) were included (Table S2). Feathers were sampled from museum skins, lodged with the Western Australian Museum (WAM) between 1920 and 1975, and cover a significant geographical area of their current distribution (Fig. 2).

DNA extraction and microsatellite genotyping

For the historical samples, all work was carried out in a purpose-built ancient DNA laboratory (Murdoch University, Perth, Australia) to prevent contamination. DNA was extracted with QIAamp DNA Micro kits (Qiagen), following a modified protocol from Bush (2007). A real-time quantitative PCR (qPCR) assay was employed to assess the extracts for quality and quantity of nuclear DNA prior to microsatellite genotyping. Genetic profiles were generated from 19 microsatellite markers detailed in White *et al.* (2009). DNA fragments were separated on an Applied Biosystems 3730 DNA Analyser and sized by co-running a size standard (Genescan-500 LIZ; Applied Biosystems). DNA fragments were scored manually with GeneMarker v1.8 (Soft Genetics). To minimise the effects of allelic drop-out often encountered with degraded templates, the historical samples were DNA profiled and repeated in triplicate to ensure reproducibility and the accuracy of allele scores. Following Bourke *et al.* (2010), homozygotes were accepted when all three replicates were homozygous, whereas heterozygotes were accepted when two of three replicates were heterozygous.

For the contemporary samples, the sub-adult/adult birds could potentially be previously sampled nestlings, therefore the data were examined for identical multi-locus genotypes in GenAlEx v6.41 (Peakall & Smouse, 2006).

Genetic structure, differentiation and gene flow

We used a Bayesian statistical analysis in the program STRUCTURE v3.2.1 (Pritchard *et al.*, 2000; Manel *et al.*, 2005; Hubisz *et al.*, 2009) to investigate the spatial genetic substructure of white-tailed black-cockatoos throughout their range. The Markov Chain Monte Carlo simulation (MCMC) was run for 1,000,000 iterations, after a burn-in of 50,000 iterations, for values of K (proposed number of populations) from 1 to 12, with 20 replicates for each K value. An admixture model assuming correlated allele frequencies and shared ancestry was implemented (White *et al.*, 2011), as the birds are unlikely to constitute completely isolated genetic units in the investigated area (Fig. 2). The most likely number of genetic units (represented by highest ΔK) was calculated with the method defined by Evanno *et al.,* (2005) utilizing the program StructureHarvester v0.6.1 (Earl, 2011). In addition, we compared corrected estimates of the admixture proportions (Q) using a likelihood ratio test, and evaluated the probability of assignment to each genetic unit, cluster (K) and the alpha value. Individuals were assigned to a genetic unit when Q was $\geq$ 0.75. STRUCTURE was run separately on the nestlings (n = 347), the sub-adult/adult (n = 313) birds, and the entire contemporary dataset (n = 660) to investigate if the different species groups and life history stages were influencing the overall genetic structure differently. Lastly, the historical genotypes were pooled with the contemporary dataset and analysed to test if STRUCTURE could uncover temporal changes in allele frequencies.

Detection of demographic change – BOTTLENECK and MSVAR analysis

We investigated the occurrence of recent demographic population changes using the program BOTTLENECK (Piry *et al.,* 1999). BOTTLENECK estimates are based on the detection of heterozygote excess compared to the expected mutation-drift equilibrium. Depending on the magnitude of the bottleneck, the method can register transient fluctuations in population size as far as 0.2**Ne* generations back in time (Sunnucks, 2011). A two-phase mutation model was applied with 95% single-step and 5% multiple-step mutations, as recommended by Piry *et al.,* (1999). Significance of deviation was tested using the Wilcoxon sign-rank test with 1000 iterations.

We used MSVAR v1.3 (Beaumont, 1999; Storz & Beaumont, 2002) to estimate the posterior probability distributions for the rate of population size change ($r = N_0/N_1$), the timing of when the change occurred ($t_f = t_a/N_0$ - a measure scaled by N_0), and the scaled mutation rate θ. In the analysis of 10 microsatellite loci and 100 white-tailed black-cockatoo nestlings from the western region, fifteen independent chains were run, with different starting demographic histories (Table S3). Wide uniform priors were used between -5 and 5 (on a $\log_{10}$-scale) for θ, r, and t_f. The data outputs from the MSVAR runs were interpreted using scripts written for purpose using the program R (R Development Core Team, 2008). We discarded the initial 10-50% of sampled points for each run to ensure the distributions were not influenced by the starting values. The total number of iterations was always > 10^9 and a total of between 10,000-50,000 points were collected for each run (using a cluster array at http://www.ivec.org/). Convergence of chains were checked visually and more formally using the Gelman-Rubin statistic (R library, boa; Smith 2005) and was measured for Log_{10} (θ, r, t_f). We used Gelman *et al*. (2004) rule of thumb, which suggests that values of the multivariate Gelman

and Rubin's convergence diagnostic between 1.0 and 1.1 indicate reasonable convergence, whereas values > 1.1 indicate poor convergence. Approximate posterior densities for 0.1, 0.5 and 0.05 (highest posterior density limits; HPD) were calculated and graphically represented (R library: boa, hdrcde and Locfit). We also used a method by Storz & Beaumont (2002) to quantify the effective population size of the current (N_0) and ancestral (N_1) population and the time (T) since the population changed. The prior distributions of μ, N_0, N_1, and T are assumed to be log-normal. As such, we used wide uninformative priors, multiple runs (at least three independent runs as a test of convergence, with differing starting seeds) and varying hyperprior parameters (Table S4). Again, the total number of iterations was always > 10^9 and 10,000-50,000 points were collected for each run. Hyperprior values varied (Table S4), however in general, different runs of values of N_0, N_1, and T had means of between 4.0 and 6.0, and with μ we set a mean of -3.5, with a standard deviation of 0.25. This value allows for a mutation rate of between 10^{-4} to 10^{-3} with reasonable support, and widely used in demographic analyses (Storz & Beaumont, 2002). We considered the generation time for white-tailed black-cockatoos to be 15 years (Saunders & Ingram, 1998; Cale, 2003).

Table S1. Source of contemporary genetic samples included in this study. A total of 660 white-tailed black-cockatoo samples collected between 1996 and 2010.

Shire/District in Western Australia	Location	Number of nestlings	Shire/District in Western Australia	Location	Number of sub-adult/adult
Borden	Private Property	22	Albany	Albany	2
Bunbury	Dalyellup	2	Bindoon	Bindoon	1
Chittering	Captive-rearing program	13	Borden	Borden	3
Coorow	Private Property A	75	Bunbury	Bunbury	1
Coorow	Private Property B	63	Busselton	Busselton	1
Dandaragan	Cataby Reserve	38	Chittering	Chittering	1
Dandaragan	Captive-rearing program	9	Coorow	Coorow	12
Dandaragan	Marchagee Track	4	Dandargan	Dandaragan	1
Dandaragan	Minyulo Nature Reserve	2	Denmark	Denmark	2
Gingin	Captive-rearing program	6	Esperance	Esperance	4
Katanning	Badgebup Nature Reserve	13	Northam	Gidgegannup	2
Katanning	Kwobrup Nature Reserve	5	Gingin	Gingin	2
Lake Grace	Dragon Rocks Nature Reserve	3	Ravensthorpe	Hopetoun	111
Lake Grace	Newdegate	1	Dandaragan	Jurien Bay	1
Lake Grace	Private property	6	Katanning	Katanning	1
Moora	Township	42	Manjimup	Manjimup	7
Moora	Private property	5	Stirling Ranges	Manypeaks	1
North of Perth	Captive-rearing program	18	Mundaring	Mundaring	1
Ravensthorpe	Cocanarup Timber Reserve	5	Mundijong	Mundijong	1
Stirling Ranges	Moingup Springs	1	Esperance	Munglinup	37
Stirling Ranges	Private property	2	Nannup	Rehabilitation Centre	15
Tambellup	Dartnall Siding	1	Swan coastal plain	Perth Zoo Vet	30
Tambellup	Tunney Reserve	4	Swan coastal plain	Perth Metro	53
Tambellup	Water Reserve	1	Swan	Pickering Brook	13
Tambellup	Private property	6	Plantagenet	Plantagenet	1
			Albany	Wellstead	1
			Swan coastal plain	Rehabilitation Centre	8

Table S2 Source of historical genetic samples included in this study. A total of 24 long- and short-billed white-tailed black-cockatoos samples, listed in chronological order, collected between 1920 and 1976 and lodged with the Western Australian Museum (WAM).

Shire/District in Western Australia	Location in Western Australia	Long- or Short-billed form	Date collected	WAM accession no.
Stirling Ranges	Moir Pass	Short-billed	21 December 1920	A1926
Stirling Ranges	Moingup Pass	Short-billed	3 November 1920	A1925
Stirling Ranges	Moingup Pass	Short-billed	9 November 1920	A1923
Ravensthrope	Hopetoun	Short-billed	23 December 1946	A6400
Lake Grace	Kukerin	Short-billed	9 January 1949	A6554
Wandering	Bannister	Short-billed	17 April 1954	A7357
Plantagenet	Mount Barker	Long-billed	28 November 1959	A9118
Plantagenet	Mount Barker	Short-billed	28 November 1959	A9119
Pemberton	Pemberton	Long-billed	3 June 1960	A9123
Pemberton	Pemberton	Long-billed	3 June 1960	A9126
Denmark	Denmark	Long-billed	29 December 1962	A9122
Unknown	Unknown	Long-billed	4 April 1963	A9121
Katanning	Kwobrup Reserve	Short-billed	1971	A18037
Mount Helena	Mount Helena	Short-billed	1 July 1971	A12465
Mundaring		Long-billed	20 July 1971	A12466
Mundaring	Water catchment	Long-billed	20 July 1971	A15973
Mundaring		Short-billed	17 May 1972	A18031
Gnangara	Pine plantation	Short-billed	3 June 1972	A15975
Mundaring	Helena Brook plantation	Long-billed	20 June 1972	A18019
Mundaring	Watsons Road	Short-billed	20 June 1972	A18029
Mundaring	Water catchment	Short-billed	27 July 1972	A15974
Mundaring	Watsons Road	Short-billed	31 July 1972	A18024
Mundaring	Stoneville	Short-billed	28 August 1975	A18025
Harvey	Wokalup Research Station	Long-billed	16 October 1975	A18014

Table S3. Starting values for the MCMC (Markov Chain Monte Carlo) simulations of program MSVAR v1.3 (Beaumont, 1999). Two independent runs were carried out with different random seeds. Identical starting parameters (but different starting seeds) were run for both linear and exponential models of growth.

MCMC run	Starting values			Steps	Thinning	Iterations
	θ	r (N_0/N_1)	T_f (t_a/N_0)			
1	0.1	0.1	0.1	10^6	10^3	10^9
2	1	0.1	1	10^6	10^3	10^9
3	10	0.1	10	10^6	10^3	10^9
4	0.1	1	0.1	10^6	10^3	10^9
5	1	1	1	10^6	10^3	10^9
6	10	1	10	10^6	10^3	10^9
7	0.1	10	0.1	10^6	10^3	10^9
8	1	10	1	10^6	10^3	10^9
9	10	10	10	10^6	10^3	10^9

Table S4. Parameters and starting values for the MCMC (Markov Chain Monte Carlo) simulations of program MSVAR v1.3 (Storz & Beaumont, 2002). Three independent runs were carried out with identical starting parameters (but different starting seeds) and for both linear and exponential models of growth.

MCMC run	Starting values (mean, variance) for				Hyperpriors (α, σ, β, τ) for				Run lengths		
	Log(N_0)	Log(N_1)	Log(μ)	Log(T)	Log(N_0)	Log(N_1)	Log(μ)	Log(T)	Steps	Thinning	Iterations
1	4, 1	4, 1	-3.5, 1	3, 1	3.4, 2, 0, 0.5	5, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	2, 3, 0, 0.5	10^5	10^4	10^9
2	4, 1	4, 1	-3.5, 1	3, 1	3.4, 2, 0, 0.5	4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	3, 3, 0, 0.5	10^5	10^4	10^9
3	4, 1	4, 1	-3.5, 1	3, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	4, 3, 0, 0.5	10^5	10^4	10^9
4	4, 1	4, 1	-3.5, 1	3, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	2, 3, 0, 0.5	10^5	5×10^4	5×10^9
5	4, 1	4, 1	-3.5, 1	3, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	3, 3, 0, 0.5	10^5	5×10^4	5×10^9
6	4, 1	4, 1	-3.5, 1	3, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	2, 3, 0, 0.5	10^5	2×10^4	2×10^9
7	4, 1	4, 1	-3.5, 1	3, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	3, 3, 0, 0.5	10^5	2×10^4	2×10^9
8	4, 1	4, 1	-3.5, 1	3, 1	5, 2, 0, 0.5	5, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	2, 3, 0, 0.5	10^5	10^4	10^9
9	4, 1	4, 1	-3.5, 1	3, 1	5, 2, 0, 0.5	4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	5, 2, 0, 0.5	10^5	10^4	10^9
10	4, 1	4, 1	-3.5, 1	3, 1	5, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	5, 2, 0, 0.5	10^5	10^4	10^9
11	4, 1	4, 1	-3.5, 1	3, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	5, 3, 0, 0.5	10^5	10^4	10^9
12	4, 1	4, 1	-3.5, 1	1, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	2, 2, 0, 0.5	10^5	10^4	10^9
13	5, 1	5, 1	-3.5, 1	2, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	5, 3, 0, 0.5	10^5	10^4	10^9
14	6, 1	6, 1	-3.5, 1	4, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	5, 3, 0, 0.5	10^5	10^4	10^9
15	6, 1	6, 1	-3.5, 1	5, 1	3.4, 2, 0, 0.5	3.4, 3, 0, 0.5	-3.5, 0.25, 0, 0.5	5, 3, 0, 0.5	10^5	10^4	10^9

Table S5. The output from the STRUCTURE analyses are presented. The Markov Chain Monte Carlo simulation (MCMC) was run for 1,000,000 iterations, after a burn-in of 50,000 iterations, for values of *K* from 1 to 12, with 20 replicates for each *K* value. The most likely number of genetic units (represented by highest Δ*K*) was calculated with the method defined by Evanno *et al*. (2005) utilizing the program StructureHarvester v0.6.1 (Earl, 2011).

Historical and Contemporary data set (*n* = 684)						
K	**Reps**	**Mean LnP(K)**	**Stdev LnP(K)**	**Ln'(K)**	**|Ln"(K)|**	**Δ*K***
1	20	-41899.1950	0.0686	-	-	-
2	20	-40878.4050	0.5781	1020.790000	433.255000	749.463730
3	20	-40290.8700	2.2373	587.535000	210.775000	94.210883
4	20	-39914.1100	3.2143	376.760000	187.035000	58.189114
5	20	-39724.3850	26.3945	189.725000	29.025000	1.099662
6	20	-39505.6350	14.2805	218.750000	115.915000	8.117008
7	20	-39402.8000	109.5035	102.835000	22.510000	0.205564
8	20	-39277.4550	7.9408	125.345000	54.420000	6.853215
9	20	-39206.5300	10.7855	70.925000	23.215000	2.152423
10	20	-39158.8200	33.3142	47.710000	25.860000	0.776245
11	20	-39085.2500	80.6649	73.570000	25.772632	0.319502
12	19	-39037.4526	12.7992	47.797368	-	-
Contemporary data set (*n* = 660)						
1	20	-40278.545	0.0887	-	-	-
2	20	-39259.265	0.5976	1019.28	462.29	773.571342
3	20	-38702.275	2.4188	556.99	170.415	70.455554
4	20	-38315.7	3.5076	386.575	275.57	78.564009
5	20	-38204.695	62.9378	111.005	111.765	1.775802
6	20	-37981.925	3.3829	222.77	126.345	37.348025
7	20	-37885.5	5.6649	96.425	64.34	11.357572

8	20	-37853.415	19.818	32.085	5.91	0.298214
9	20	-37815.42	44.3959	37.995	45.705	1.029486
10	20	-37731.72	64.1395	83.7	15.795	0.24626
11	20	-37663.815	12.1542	67.905	113.015	9.298467
12	20	-37708.925	90.0592	-45.11	-	-

Table S6. Contemporary (n = 660) and historical (n = 24) white-tailed black-cockatoo sample groups: number of individuals (N), number of alleles per locus (N_A), mean observed (H_O) and expected (H_E) heterozygosity, allelic richness (Ar), and private allelic richness (PAr) averaged over 19 loci.

Locus	**Contemporary (1996-2010)**						**Historical (1920-1975)**					
	N	N_A	H_O	H_E	***Ar***	***PAr***	***N***	N_A	H_O	H_E	***Ar***	***PAr***
p*Cl*A119	638	17	0.66	0.68	6.29	2.58	23	10	0.74	0.65	6.67	2.97
p*Cl*A139	634	11	0.70	0.74	5.20	1.31	16	7	0.56	0.75	5.75	1.87
p*Cl*D108	602	9	0.39	0.82	5.79	1.08	9	5	0.33	0.75	5.00	0.29
p*Cl*A125	646	15	0.85	0.88	8.30	3.70	24	10	0.79	0.86	7.92	3.31
p*Cl*A9	656	12	0.66	0.66	5.40	1.10	23	7	0.78	0.75	5.43	1.12
p*Cl*A128	600	12	0.74	0.79	5.61	2.27	18	7	0.78	0.72	5.46	2.12
p*Cl*A138	648	8	0.62	0.74	4.96	2.09	20	3	0.45	0.51	2.96	0.09
p*Cl*A118	655	8	0.71	0.71	4.27	1.20	24	4	0.75	0.73	3.99	0.92
p*Cl*5	641	9	0.53	0.56	4.21	0.78	21	5	0.48	0.57	4.10	0.63
p*Cl*3	654	10	0.66	0.72	5.26	2.02	24	8	0.71	0.76	5.73	2.49
p*Cl*D7	639	11	0.68	0.78	5.89	1.68	24	6	0.50	0.69	4.85	0.65
p*Cl*D105	624	11	0.68	0.74	5.47	1.44	24	6	0.58	0.73	5.10	1.06
p*Cl*D118	642	9	0.40	0.79	5.93	0.65	20	7	0.25	0.83	6.48	1.20
p*Cl*8	652	9	0.59	0.61	4.27	0.36	23	6	0.61	0.69	5.26	1.35
p*Cl*D122	648	9	0.79	0.79	5.85	0.82	24	8	0.71	0.78	6.17	1.15
p*Cl*D114	634	6	0.45	0.48	3.93	1.04	21	4	0.43	0.45	3.49	0.60
p*Cl*D112	612	6	0.61	0.68	3.78	0.22	14	4	0.43	0.61	3.84	0.28
p*Cl*A105	655	12	0.75	0.76	5.12	3.78	23	4	0.78	0.74	3.99	2.65
p*Cl*D109	635	7	0.66	0.68	4.31	0.44	16	5	0.62	0.72	4.53	0.66
Mean	**637.63**	**10.05**	**0.64**	**0.72**	**5.26**	**1.50**	**20.57**	**6.10**	**0.59**	**0.70**	**5.09**	**1.34**

Table S7. Pairwise measures of genetic differentiation (F_{ST} above the diagonal) and indirect estimates of the number of migrants per generation (*Nm* below the diagonal).

	C. latirostris nestlings	*C. latirostris* sub-adult/adult	*C. baudinii* sub-adult/adult
C. latirostris nestlings	-	0.013 $P = 0.001$	0.045 $P = 0.001$
C. latirostris sub-adult/adult	19.67	-	0.048 $P = 0.001$
C. baudinii sub-adult/adult	5.25	5.01	-

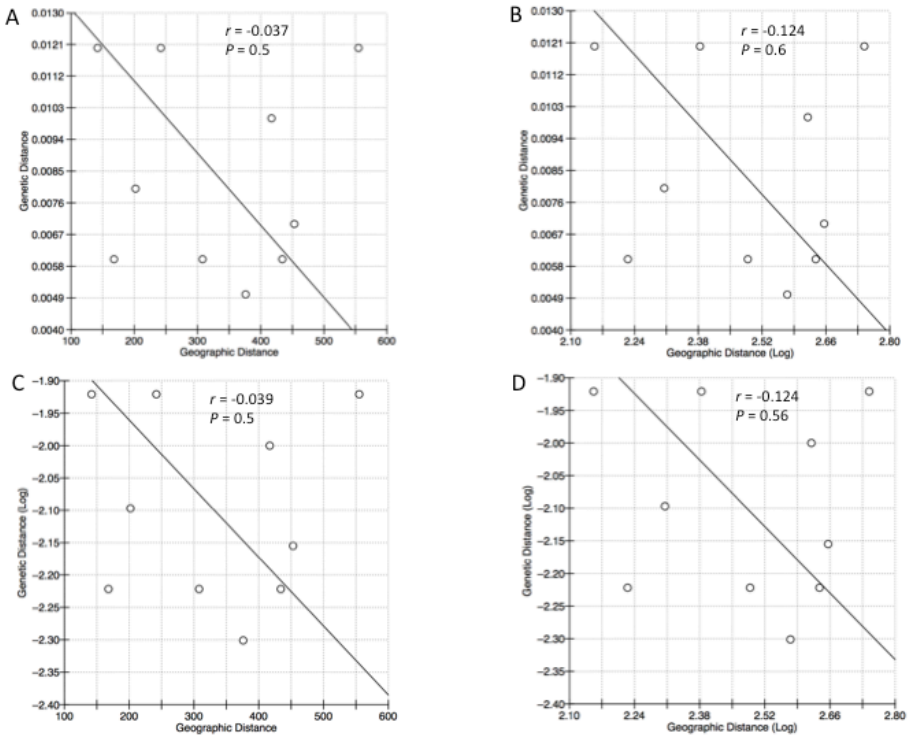

Figure S1. Tests for genetic isolation-by-distance of the white-tailed black-cockatoo sample groups (nestlings, adult/sub-adult, and morphotypes) east and west of the Avon Wheatbelt, as inferred using multi-locus genetic distance estimates of $F_{ST}/(1-F_{ST})$ and geographical distance (kilometres), inclusive of the logarithm. Pearson correlation (r) and probability (P) values are shown for each Mantel test; A - correlation between genetic distance and geographic distance; B – matrix correlation between genetic distance and log (geographic distance); C – matrix correlation between log (genetic distance) and geographic distance; and D – matrix correlation between log (genetic distance) and log (geographic distance).

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