Case report for Forensic Science International: Genetics

Title: Application of STR markers in wildlife forensic casework involving Australian black-cockatoos (*Calyptorhynchus* spp.)

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Abstract

Parrots and cockatoos are highly prized aviary birds and the demands for such species has fuelled their illegal trade and harvest from the wild. Here we report on three forensic case studies involving black-cockatoos (*Calyptorhynchus* spp.) endemic to Australia. These cases involve suspected poaching and illegal killing of endangered red- and white-tailed black-cockatoos. Through the prior development of 20 polymorphic microsatellite loci and population databases for white- and red-tailed black-cockatoos, the tools are available to conduct high-resolution paternity and individual identity testing. In one case, we matched a red-tailed black-cockatoo nestling to a tree hollow from which it was poached through the use of DNA from eggshell recovered from the nest. For the second case, we utilized our provenance population database (nest sites), and identified the kinship and geographic origin of a white-tailed black-cockatoo, of which was illegally harvested from the wild. The third case determined the number individual white-tailed black-cockatoos allegedly shot at a fruit grower's orchard from body part remains. These genetic investigations highlight the significance and statistical confidence of DNA profiling and associated databases for endangered taxa, such as exotic birds. Our cockatoo population databases are the first of their kind in Australia, and demonstrate the efficacy of such approaches to identify such illegal activity. With a robust set of genetic markers and methodologies in place, we aim to broaden our population databases to include other cockatoo species of conservation concern.

Keywords: wildlife forensics, poaching, population database, feathers, eggshell

1. Introduction

Parrots and cockatoos (order Psittaciformes) are recognized globally for their extraordinary plumage, mimicry ability and charismatic character. It is these features that have fuelled their exploitation and capture from the wild [1]. Estimates of the number of birds extracted from the wild are difficult to quantify – the international estimates ranged from ~7.5 million birds per annum during the peak of trade in the 1970s to around 2 to 5 million individuals per annum in the 1980s [2], with parrots accounting for half of the 519 species of birds traded from 1991 to 1996 [2]. Captive breeding is a major source of individuals to supply aviculture markets, although only a small number of species are sourced from this industry [2]. The total impact of the

illegal trade in birds is particularly challenging to quantify in numbers due to its clandestine nature, and the cumulative impact of the mortality rates during capture, transport and pre- and post-sale [2-4]. The detrimental impacts of such activities are having a measurable effect on cockatoos and parrots in the wild, both in terms of biodiversity and population number [2,3]. Spix's macaw (*Cyanopsitta spixii*) is one of the most recent cases of extinction in the wild, caused by illegal harvest [1,3]. The rarity of this bird in captivity, fuelled by demand by collectors has caused prices to rise to ~AUD$20,000 per bird [1]. Clearly, there are considerable monetary motivations for taking part in the illegal bird trade, especially considering that prosecutions are rare and the penalties are not particularly harsh [5].

Psittaciformes contain over 370 species, which are mainly concentrated in the Southern Hemisphere [6]. Combinations of anthropogenic induced habitat loss and modification, poaching and illegal trade are significant threats to these birds. Over 20% of parrots and cockatoos are of conservation concern, 85 species are listed as critically endangered, endangered or vulnerable, and 19 species as extinct by the International Union for the Conservation of Nature [7]. In Australia there are five endemic species of black-cockatoo (*Calyptorhynchus* spp.), and the two endangered white-tailed black-cockatoo (WTBCs) of Western Australia have been particularly detrimentally affected by anthropogenic activities [7]. White-tailed black-cockatoos have declined ~50% in population number as a result of habitat loss [8,9], and they show evidence of population substructure and gene exchange between the two forms (short- and long-billed), possibly as a result of severe land clearing for agriculture [6,10]. Taxonomic amendment is currently under review based on recent genetic data [6,10]; therefore the long- (*C. baudinii*) and short-billed (*C. latirostris*) forms, currently accepted in the Australian checklist of birds [11], will be collectively referred to as WTBCs.

A dichotomy exists for WTBCs amongst some of the West Australian community. On one hand, illegal shooting of WTBCs regularly occurs, with estimates of the number of WTBCs shot annually in the hundreds (P. Mawson, personal communication) despite the fact that WTBCs have had protected species status since 1989. Conflicts with humans occur due to the birds feeding on fruit and nut crops [12], which have in select areas replaced native foraging habitat. Illegal collection of eggs and nestlings

from the wild to supply aviculture demand was historically common [3,13,14], as breeding in captivity is notoriously difficult and has been recorded infrequently [14,15]. On the other hand, in 1996 a WTBC captive-rearing program was established to increase the supply of the short-billed form in captivity. The program involved the sustainable harvest of eggs and nestlings from the wild [13]. The aim of this program was to provide the short-billed form for the Australian domestic aviculture market in order to reduce the value of these birds on the legal market, and curtail the financial incentives for illegal take from the wild. All captive-reared birds were micro-chipped, and biological samples taken for inventory of genetic diversity maintained in captivity [13,16].

One area of conservation genetics that has long been recognized, is the development of analytical techniques capable of providing DNA evidence to assist in conservation law enforcement, commonly termed 'wildlife DNA forensics' [17]. There is an increasing forensic use of animal DNA to determine either; 1) the identity of samples to a genus and/or species level; or 2) assigning samples to an individual or to kin [18]. Species identification with the use of DNA is now widely applied [4,18-24], and assigning samples to an individual, population, or geographic origin is gaining momentum [25-29].

Unlike human forensic DNA profiling and analysis, wildlife DNA forensic markers used to profile avian samples, target microsatellite (STR) loci that are either developed for the species of interest, or taken from an open access database such as GenBank, and examined for specificity (cross-species amplification) [18,30]. Then the development of a DNA profile or population database for the species of interest is conducted, and when practicable, should contain an adequate number of samples and be representative of the population(s) in the wild. Often 200 members are the *de facto* standard for a population database [18], although the size of databases is dependent on the number of potential contributors and locus diversity levels [18]. There are numerous advantages and applications of such databases, although there are few such databases in existence for wild animals [18,29,31]. From a forensic genetic perspective, identifying the geographic origin of a sample is equivalent to identifying its reproductive population of origin [18,28]. Often it is necessary to demonstrate from a body part (e.g. horn, tusk, bone, skin) or by-product, such as eggshell, if the

sample(s) originated from a specific individual, or from similar body parts, if they were derived from a number of individuals, such as shark fins in prepared food. Of equal importance is the regulation of the legal trade and harvest of species, to ensure that illegally obtained wildlife cannot be laundered into a legitimate supply chain. In Western Australia there are many suspected instances of illegal activity involving cockatoos. Together with the assistance of the Western Australian Department of Environment and Conservation (DEC) we are now able to successfully implement DNA profiling technologies with the aim of increasing the probative value of evidence presented in wildlife DNA forensic cases.

2. Case history

2.1 Red-tailed black-cockatoo case 1

Suspected poaching from the wild, Property A

On December 9th of 2008 an alert member of the public photographed a man on road reserve vested in the Local Government Authority in Morawa (310km NNE of Perth), Western Australia climbing a tree with a ladder (Fig. 1a). The licence plate number of the man's vehicle was included in the photographs. The photographs were sent to the Nature Protection Branch, DEC. A search warrant was issued for the address (property A) listed under the vehicle registration via details sourced from the licence plate number. On December 19th of 2008, four wildlife officers from DEC executed a search warrant at Property A and discovered four galahs (*Eolophus roseicapillus*) and a red-tailed black-cockatoo (*C. banksii*; RTBC) nestling ~7-8 weeks of age (Fig. 1b). A nestling is defined here as a bird that has not reached the age where it has the ability to fly. Upon inspection of the tree, wildlife officers recovered eggshell from inside the hollow (Fig. 1c), and retained it as evidence. The nestling (Fig. 1b) and four galahs were seized from Property A as evidence and placed into captive-care.

2.2 White-tailed black-cockatoo case 2

Suspected poaching from the wild, Property B

Upon inspection of Property A (case 1 above), wildlife officers heard the distinct sounds of black-cockatoo nestlings within close range. A wildlife officer looked over the fence of Property A into the backyard of Property B (Fig. 2a) and saw numerous black-cockatoos in aviaries. Such breeding success is unusual and wildlife officers subsequently inspected Property B and seized 11 RTBCs (nestlings and adults), and

three WTBCs (2 nestlings and 1 adult) (Fig. 2b). The 15 black-cockatoos were placed into captive-care.

2.3 White-tailed black-cockatoo case 3

Suspected illegal shooting of White-tailed black-cockatoos

On March 19th of 2009, a member of the public submitted four carcases of WTBC to the Nature Protection Branch, DEC. The carcases were found and collected from an apple orchard located on the outskirts of Perth, Western Australia. Wildlife officers inspected the apple orchard where the four carcases were allegedly sourced. Upon inspection of the apple orchard wildlife officers found bird remains (feathers and body parts); these remains were collected as evidence (Fig. 3).

The above three cases involved the alleged poaching and illegal killing of black-cockatoos in Western Australia. With the use of molecular markers designed specifically for WTBCs that have high cross-species utility in other cockatoo species [32], and utilizing local population genetic database(s) [32], our investigations were to test the following hypotheses:

- Red-tailed black-cockatoo case 1 – determine if the DNA profile from a RTBC nestling seized by wildlife authorities match the DNA profile from eggshell recovered from a tree hollow from which it was allegedly poached;
- White-tailed black-cockatoo case 2 - identify the reproductive population of origin for three WTBCs seized from a private property;
- White-tailed black-cockatoo case 3 – determine the number of individual WTBCs from remains collected at a fruit-growers orchard.

3. Materials and methods

Feathers (approx., 4-8) were collected from four black-cockatoos held in captive-care and 13 deceased black-cockatoos (Table 1), and stored in 20% dimethylsulphoxide saturated with sodium chloride. Eggshell from a tree hollow was stored in a clean, dry sample container. DNA from the feathers was extracted using Chelex®100 (supplementary information), and DNA from the eggshell was extracted following the protocol of Oskam et al. [33]. DNA extracts, prior to genotyping, were quantified (qPCR) for nuclear DNA yield, and normalised (supplementary information). Relative quantification using qPCR enables the identification of inhibition and low copy

number DNA samples - as such it represents an important step in quality control. For genetic profiling of red- and white-tailed black-cockatoos we used 17-19 microsatellite loci [32]. The primers used to amplify polymorphic DNA were tested to ensure specificity and reproducibility [10,18,32]. The multiplex assays contained internal controls, as seven locus are routinely replicated in two of the four multiplex assays for every DNA profile generated. DNA fragments were separated on an Applied Biosystems 3730 DNA Analyser. Size was determined by co-running a size standard (Genescan™-500 LIZ™, Applied Biosystems, Foster City) and fragments were scored manually with the aid of GeneMarker v1.8 Software (Soft Genetics).

3.1 Red-tailed black-cockatoo case 1

In total, one sample from the RTBC nestling and one sample from the eggshell were processed. Probabilities of identity (P_{ID} and $\boldsymbol{P_{IDsibs}}$) and random match probabilities (RMP and $\mathbf{RMP_{sibs}}$) in the RTBC were calculated for 17 loci, using the allele frequencies database that contained 30 individuals, in the program GenAlEx v6.4 [34]. These estimates were used to show the frequency, with a conservative upper bound, at which a particular DNA profile would be expected to occur in the population. The genetic profiles of B08-750 and B08-765 (Table 1) were examined for matching genotypes. Population size and substructure for red-tailed black-cockatoos were taken into account with a conservative theta ($\theta = 0.03$) correction factor following the National Research Council (NRC II) recommendation.

3.2 White-tailed black-cockatoo case 2

In total, three WTBC samples (B08-753, B08-754, B08-763) were processed (Table 1). Genetic parameters for the reference population such as allele frequencies, deviation from Hardy-Weinberg proportions, and linkage equilibrium have been previously described by White et al. [10,32]. The reference population database contained genetic profiles from 347 individual WTBC nestlings of known provenance. Probabilities of identity (P_{ID} and $\boldsymbol{P_{IDsibs}}$) and random match probabilities (RMP and $\mathbf{RMP_{sibs}}$) in the WTBC were calculated for 20 loci using the allele frequencies database. To account for population substructure a theta ($\theta = 0.02$) correction factor was used based on empirical estimates for white-tailed black-cockatoos. In order to identify the reproductive population of origin we applied a relatedness (kinship) analysis in the program ML-Relate [35] and GenAlEx v6.4 [34].

The kinship analyses fall into two major classes, a methods-of-moments (MM) estimator that is designed for estimating the relatedness (r) between a pair of individuals which is a continuous quantity (0 to 1) defined in terms of identity by descent (IBD), and a maximum likelihood (ML) estimator that determines the likelihood of a pair of individuals falling into a particular type of relationship category (e.g. full-sibling vs half-sibling). GenAlEx was used to perform initial pair-wise estimates between all pairs of WTBC using the MM estimator of Queller and Goodnight [36], and ML-Relate was used to calculate the ML estimates and test the relationships with a statistical approach for two *a priori* hypotheses with confidence sets. For example, the likelihood ratio test examines the putative relationship such as full-siblings against the alternative relationship such as half-siblings with a confidence interval set by the investigator. If the *P*-value is found to be small, the putative relationship fits the data significantly better than the alternative relationship [35].

3.3 White-tailed black-cockatoo case 3

In total 13 WTBC samples (B09-128, B09-130 to B09-141) were processed, inclusive of the four carcases submitted to DEC (Table 1). The genetic profiles were examined for the number of unique genotypes, and the 'matching genotype' function within GenAlEx was used to examine if any of the WTBCs from the orchard had been previously genotyped using the population database as a reference.

4. Results and discussion

4.1 Red-tailed black-cockatoo case 1

DNA was successfully recovered from samples B08-750 and B08-765 (Table 1). Complete 17-locus genotypes were obtained (Table S1). Multilocus genotypes of B08-750 and B08-765 were found to be identical, except for locus p*Cl*D122, in which a homozygous profile was obtained for B08-765 (Table S1) – we suspect this was a single incidence of allelic dropout. Probability of identity (P_{ID}) in the RTBC population was calculated using the allele frequencies in the database (Table 2). P_{ID} values for each locus are presented in Table S1. The probability (P_{ID}) that two individuals selected at random from the RTBC population would have the same multi-locus genotype was 1.9E-16, and P_{IDsibs} of 1.2E-6 at a 17-locus combination. The random match probability for the DNA profile of sample B08-765 was estimated at 2.57E-12 (Table S2) that accounted for population substructure and the homozygous

locus p*Cl*D122. Analysis of the nestling (B08-750) and eggshell (B08-765) indicates that if this nestling was not hatched from the eggshell then the DNA profile must match by chance. Our estimates show that the chance of obtaining these matching profiles, if the eggshell came from a random RTBC that is unrelated to the nestling, is in the order of 1 in 389 billion (2.57E-12), based on the alleles present in sample B08-765 (Table S2). Therefore we concluded that the RTBC nestling (B08-750) was hatched from the eggshell (B08-765) part of which was recovered from the tree hollow.

Under the *Wildlife Conservation Act 1950*, it is illegal to take or possess protected fauna without the authority of a licence. The resident of Property A pleaded guilty to taking protected fauna, namely four galahs and one RTBC without the authority of a licence. He was fined AUD$1000 in addition to court costs, forfeiture of his ladder and brooder. Due to the age of the nestling when it was taken from the wild, and the daily human interaction required for its care, it could not be released back into the wild as it had become imprinted (early social attachment to humans). The four galahs and RTBC remain in captive-care.

4.2 White-tailed black-cockatoo case 2

DNA was successfully recovered from samples B08-753, B08-754, and B08-763 (Table 1). Complete 19-locus genotypes were obtained (Table S3), and found to be unique. The P_{ID} in the WTBC population was calculated using the allele frequencies in the database (Table 2). P_{ID} values for each locus are presented in Table S4. The probability (P_{ID}) that two individuals selected at random from the WTBC population would have the same multi-locus genotypes was 2.6E-20 and P_{IDsibs} of 2.4E-8 for a 20-locus combination (Table 2). Sample B08-763 was identified as a full-sibling (FS) to a nestling (B08-097) in the WTBC population database. Samples B08-763 (Table 1) and B08-097 were found to share 55% of alleles. The match probability for full-siblings was calculated for sample B08-763 that accounted for population substructure and was found to be 1.48E-07 (Table S4). Analysis of the DNA profile recovered from sample B08-763 indicates that if this WTBC is not related to B08-097 then the DNA profile must match (55% shared alleles) by chance. Our estimates show that the chance of obtaining full-sibling matching profiles, if sample B08-763 came from a random unrelated WTBC breeding pair, is in the order of 1 in 6 million (1.48E-07),

based on the alleles present in sample B08-763 (Table S4). The log likelihood of the FS relationship was high (LnL(*r*) -88.22). This FS relationship was 9.86 times more likely than a half-sibling (HS) relationship, and 17.51 times more likely than no genetic relationship. The likelihood ratio test for the *a priori* relationships (FS or HS) showed the FS (putative relationship) fitted the data significantly better ($P = 0.03$) than the alternative HS relationship. Samples B08-753 and B08-754 were not related to any of the 347 nestlings in the population database.

The DNA profile of the nestling (B08-097) in the population database was from Chittering, Western Australia and removed from the wild in 1996 by the Department of Environment and Conservation as part of the captive-rearing program. This bird remains in captive-care, as the owners were contacted after we made our results public and this bird had not bred in captivity. White-tailed black-cockatoos display strong pair-bonds throughout their adult life and considered as a monogamous species [37,38]. Therefore, our findings of a full-sibling relationship are consistent with the species reproductive biology. Nest site fidelity has been documented by Saunders [39, 40], who has shown that adult breeding pairs return to the same nesting area they bred the previous season. Pair-bonds can last for life, which is thought to be up to 32 years or longer [37,41]. Given we have an understanding of this species ecology and nesting behaviour, we are confident sample B08-763 was poached from the Chittering area (75km NE of Perth).

Under the Wildlife Conservation Act it is illegal to possess protected fauna without the authority of a licence. The resident of Property B pleaded guilty to unlawful possession of three WTBCs and 11 RTBCs. He was fined AUD$3000 for the WTBCs and AUD$1500 for the RTBCs, in addition to court costs and a forfeiture of his aviaries. These birds remain in captive-care due to imprinting.

4.3 White-tailed black-cockatoo case 3

DNA was successfully recovered from 13 samples (B09-128, B09-130 to B09-141) listed in Table 1. Complete 19-locus genotypes were obtained (Table S2), and all 13 DNA profiles were found to be unique when compared to the 347 nestlings in the WTBC population database. There were concerns raised by wildlife officers in regards to the four carcases that were submitted to the Department of Environment

and Conservation, which initiated the inspection of the orchard. The concerns were that the four birds submitted might be the source of some of the subsequent material collected from the orchard upon inspection. Our results clearly show that in addition to the four DNA profiles from the four carcases, a further 9 DNA profiles were recovered. Therefore, more than four individual birds were identified from the carcasses and feathers obtained from the orchard, as a total of 13 unique WTBC DNA profiles were generated. The four carcases submitted that initiated the investigation were not the source of the other remains collected from the orchard. The 13 DNA profiles were not found to match any of the 347 individuals in the population database.

The investigation by DEC was unsuccessful in being able to positively identify the person(s) responsible for the illegal killing of WTBCs found in the orchard. Therefore, no charges were laid. Subsequent to the investigation, the orchard operators have modified their deterrent practice and now use a gas gun to assist in scaring the birds away from the fruit trees.

5. Conclusions

Two of the three cases described above indicate that it is possible to definitively determine illegal trade or harvest of live animals from the wild. This is hard, or even impossible to achieve, without the assistance of molecular techniques. DNA profiling offers a non-invasive means to monitor wild and aviary populations. We advocate, as part of the licensing agreement to maintain protected species in captivity that DNA samples should be taken with the explicit intention that they may be used to verify parentage/identity in the future if the need arises. Our study has shown that a suitable panel of microsatellite loci, and sufficient wild representatives from the WTBC population, have facilitated the reliable identification of a breeding area and show high discriminatory power for kinship testing and individual identification [42]. For RTBC, the population database contained a smaller number of representatives, although the 17-microsatellite loci provided a high degree of statistical confidence with the assignment of a sample to eggshell collected at a wildlife crime scene.

Population genotype databases are invaluable for conservation, management and protection of endangered fauna. Wasser et al. [29] have shown the applicability of

such a database for determining the locality from which elephant ivory was sourced - the challenge now is to adopt this technology for the protection of other endangered species. The black-cockatoo population database described here is the largest wildlife forensic database of its kind in Australia and could be expanded to include other cockatoo species of conservation concern. We regard our methodology and statistical resolution to be fit for purpose and broadly compliant with the recent International Society for Forensic Genetics (ISFG) recommendations regarding the use of non-human DNA in forensic genetic investigations [18]. As an enhancement of the ISFG guidelines, we routinely quantify all genomic extracts prior to genotyping to standardise the input level of DNA into multiplexes and to safeguard against issues associated with low copy number DNA [42,43].

Prior to the application of wildlife DNA forensics, the ability to determine illegal trade or harvest of live animals from the wild was difficult to prove beyond reasonable doubt. As the scale and level of sophistication of wildlife crime has increased in many parts of the world the penalties for such crimes have largely remained unchanged, and many penalties do not even match the black-market value for many species [5]. We estimate the black-market value of black-cockatoos within Australia or internationally would range from AUD$1,500 to AUD$12,500 per bird. The loss of biodiversity resulting from the removal of birds from the wild, or their extinction, is inestimable. Unfortunately, the pecuniary incentive in trafficking black-cockatoos greatly outweighs the current penalties imposed if convicted. It is our hope that the successful implementation of DNA technologies, such as described here, will start to act as a deterrent to individuals who seek to gain from the exploitation of wildlife.

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References

1. T.F. Wright, C.A. Toft, E. Enkerlin Hoeflich, J. Gonzalez Elizondo, M. Albornoz, A. RodrÌguez Ferraro, F. Rojas Su·rez, V. Sanz, A. Trujillo, and S.R. Beissinger, Nest poaching in Neotropical parrots, Conservation Biology 15 (2001) 710-720.
2. S.R. Beissinger, Trade of live wild birds: potentials, principles and practices of sustainable use, in: J.D. Reynolds, G.M. Mace, K.H. Redford, and J.G. Robinson, (Eds.), Conservation of exploited species, Cambridge University Press, Cambridge, United Kingdom, 2001, pp. 182-202.
3. D.J. Pain, T.L.F. Martins, M. Boussekey, S.H. Diaz, C.T. Downs, J.M.M. Ekstrom, S. Garnett, J.D. Gilardi, D. McNiven, and P. Primot, Impact of protection on nest take and nesting success of parrots in Africa, Asia and Australasia, Animal Conservation 9 (2006) 322-330.
4. M.L. Coghlan, N.E. White, L. Parkinson, J. Haile, P.B.S. Spencer, and M. Bunce, Egg forensics: an appraisal of DNA sequencing to assist in species identification of illegally smuggled eggs, Forensic Science International: Genetics (2011) doi:10.1016/j.fsigen.2011.06.006.
5. E. Alacs and A. Georges, Wildlife across our borders: a review of the illegal trade in Australia, Australian Journal of Forensic Sciences 40 (2008) 147-160.
6. N.E. White, M.J. Phillips, M.T.P. Gilbert, A. Alfaro-Núñez, E. Willerslev, P.R. Mawson, P. Spencer, and M. Bunce, The evolutionary history of cockatoos (Aves: Psittaciformes: Cacatuidae), Molecular Phylogenetics and Evolution 59 (2011) 615-622.
7. IUCN, *IUCN Red List of Threatened Species*. Versioin 2010.4 (2010) <http://www.iucnredlist.org>..
8. D.A. Saunders, Problems of survival in an extensively cultivated landscape: The case of Carnaby's cockatoo *Calyptorhynchus funereus latirostris*, Biological Conservation 54 (1990) 277-290.
9. D.A. Saunders and J.A. Ingram, Birds of southwestern Australia: an atlas of changes in the distribution and abundance of wheatbelt avifauna. 1995: Surrey Beatty & Sons in association with Western Australian Laboratory, CSIRO Division of Wildlife and Ecology.
10. N.E. White, D.A. Saunders, P.R. Mawson, R. Dawson, A. Alfaro-Núñez, E. Willerslev, M.T.P. Gilbert, and M. Bunce, Molecular insights into a superspecies complex (Cacatuidae: Zanda: *Calyptorhynchus*) with

implications for taxonomy, conservation management and forensic identification, IBIS in review.
11. L. Christidis and W.E. Boles, Systematics and Taxonomy of Australian Birds. CSIRO Publishing, Australia, 2008.
12. T.F. Chapman, An endangered species that is also a pest: a case study of Baudin's Cockatoo *Calyptorhynchus baudinii* and the pome fruit industry in south-west Western Australia, Journal of the Royal Society of Western Australia 90 (2007) 33-40.
13. P. Mawson, A captive breeding program for Carnaby's Cockatoo *Calyptorhynchus latirostris*, Eclectus 3 (1997) 21-23.
14. D.A. Saunders, I. Rowley, and G.T. Smith, The effects of clearing for agriculture on the distribution of cockatoos in the southwest of Western Australia, Birds of Eucalypt Forests and Woodlands: Ecology, Conservation, Management (1985) 309-321.
15. B. Blanch, White-tailed Black Cockatoos, BirdKeeper 24 (2011) 488-491.
16. E. Department of Sustainability, Water, Population and Communities, *Calyptorhynchus latirostris*, in *Species Profile and Threats Database*. 2010, Department of Sustainability, Environment, Water, Population and Communities, Canberra.
17. R. Ogden, N. Dawnay, and R. McEwing, Wildlife DNA forensics- bridging the gap between conservation genetics and law enforcement, Endangered Species Research 9 (2009) 179-195.
18. A. Linacre, L. Gusmao, W. Hecht, A.P. Hellmann, W.R. Mayr, W. Parson, M. Prinz, P.M. Schneider, and N. Morling, ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations, Forensic Science International: Genetics (2010) doi:10.1016/j.fsigen.2010.10.017.
19. C. Bellis, K.J. Ashton, L. Freney, B. Blair, and L.R. Griffiths, A molecular genetic approach for forensic animal species identification, Forensic science international 134 (2003) 99-108.
20. P.D.N. Hebert, S. Ratnasingham, and J.R. de Waard, Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species, Proceedings of the Royal Society of London. Series B: Biological Sciences 270 (2003) S96.
21. H.M. Hsieh, L.H. Huang, L.C. Tsai, Y.C. Kuo, H.H. Meng, A. Linacre, and J.C.I. Lee, Species identification of rhinoceros horns using the cytochrome b gene, Forensic science international 136 (2003) 1-11.
22. J.A. Rudnick, T.E. Katzner, E.A. Bragin, and J.A. DeWoody, Species identification of birds through genetic analysis of naturally shed feathers, Molecular Ecology Notes 7 (2007) 757-762.
23. F. Teletchea, C. Maudet, and C. Hanni, Food and forensic molecular identification: update and challenges, TRENDS in Biotechnology 23 (2005) 359-366.
24. S.K. Verma and L. Singh, Novel universal primers establish identity of an enormous number of animal species for forensic application, Molecular Ecology Notes 3 (2003) 28-31.
25. M.M. Hansen, E. Kenchington, and E.E. Nielsen, Assigning individual fish to populations using microsatellite DNA markers, Fish and Fisheries 2 (2001) 93-112.

26. S. Manel, P. Berthier, and G. Luikart, Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes, Conservation Biology 16 (2002) 650-659.
27. C.R. Primmer, M.T. Koskinen, and J. Piironen, The one that did not get away: individual assignment using microsatellite data detects a case of fishing competition fraud, Proceedings of the Royal Society B: Biological Sciences 267 (2000) 1699-1704.
28. M. Salinas, L. Altet, C. Clavel, R.M. Almela, A. BayÛn, I. Burguete, and A. S·nchez, Genetic assessment, illegal trafficking and management of the Mediterranean spur-thighed tortoise in Southern Spain and Northern Africa, Conservation Genetics 12 (2009) 1-13.
29. S.K. Wasser, A.M. Shedlock, K. Comstock, E.A. Ostrander, B. Mutayoba, and M. Stephens, Assigning African elephant DNA to geographic region of origin: applications to the ivory trade, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 14847-14852.
30. N. Dawnay, R. Ogden, J.H. Wetton, R.S. Thorpe, and R. McEwing, Genetic data from 28 STR loci for forensic individual identification and parentage analyses in 6 bird of prey species, Forensic Science International: Genetics 3 (2009) e63-e69.
31. R. Lorenzini, P. Cabras, R. Fanelli, and G.L. Carboni, Wildlife molecular forensics: Identification of the Sardinian mouflon using STR profiling and the Bayesian assignment test, Forensic Science International: Genetics (2011).
32. N.E. White, P.R. Mawson, R. Dawson, M.A. Bunce, and P.B.S. Spencer, Characterisation and cross-species utility of 20 microsatellite markers for population and forensic applications in the endangered Carnaby's Black-cockatoo, *Calyptorhynchus latirostris*, Conservation Genetics Resources 1 (2009) 341-345.
33. C.L. Oskam, J. Haile, E. McLay, P. Rigby, M.E. Allentoft, M.E. Olsen, C. Bengtsson, G.H. Miller, J.L. Schwenninger, and C. Jacomb, Fossil avian eggshell preserves ancient DNA, Proceedings of the Royal Society B: Biological Sciences 277 (2010) 1991-2000.
34. R. Peakall and P.E. Smouse, genalex 6: genetic analysis in Excel. Population genetic software for teaching and research, Molecular Ecology Notes 6 (2006) 288-295.
35. S.T. Kalinowski, A.P. Wagner, and M.L. Taper, ml-relate: a computer program for maximum likelihood estimation of relatedness and relationship, Molecular Ecology Notes 6 (2006) 576-579.
36. D.C. Queller and K.F. Goodnight, Estimating relatedness using genetic markers, Evolution 43 (1989) 258-275.
37. B. Cale, Carnaby's Black-Cockatoo (*Calyptorhynchus latirostris*) recovery plan 2000-2009, Western Australian Wildlife Management Program 36 (2003).
38. P.J. Higgins, Handbook of Australian, New Zealand & Antarctic Birds. Vol. 4, Parrots to Dollarbird. Vol. 4. Oxford University Press, 1999.
39. D.A. Saunders, Vocal Repertoire and Individual Vocal Recognition in the Short-Billed White-Tailed Black Cockatoo, *Calyptorhynchus funereus latirostris* Carnaby, Wildlife Research 10 (1983) 527-536.
40. D.A. Saunders, Breeding season, nesting success and nestling growth in Carnaby's cockatoo, *Calyptorhynchus funereus latirostris*, over 16 years at

Coomallo Creek, and a method for assessing the viability of populations in other areas, Australian Wildlife Research 13 (1986) 261-273.
41. D.A. Saunders and R. Dawson, Update on Longevity and Movements of Carnaby's Black Cockatoo, Pacific Conservation Biology 15 (2009) 72-74.
42. L.P. Waits, G. Luikart, and P. Taberlet, Estimating the probability of identity among genotypes in natural populations: cautions and guidelines, Molecular Ecology 10 (2001) 249-256.
43. M.E. Allentoft, C. Oskam, J. Houston, M.L. Hale, M.T.P. Gilbert, M. Rasmussen, P. Spencer, C. Jacomb, E. Willerslev, and R.N. Holdaway, Profiling the Dead: Generating Microsatellite Data from Fossil Bones of Extinct MegafaunaóProtocols, Problems, and Prospects, PLoS ONE 6 (2011) e16670.

Figure Legends:

Figure 1. Case 1: Three photographs taken in Morawa, Western Australia; a) an anonymous member of the public photographed a man climbing a tree, who was suspected of nest poaching, b) red-tailed black-cockatoo nestling found at Property A and placed into captive-care; c) eggshell, shown by arrow, in a nest hollow that was collected as evidence by wildlife officers. Photographs courtesy of R. Dawson, DEC.

Figure 2. Case 2: Two photographs taken in Morawa, Western Australia; a) the aviaries found in the backyard of Property B; b) two red-tailed (on left) and two white-tailed (on right) black-cockatoo nestlings found at Property B and placed in captive-care. Photographs courtesy of R. Dawson, DEC.

Figure 3. Case 3: Two photographs taken at an orchard in Western Australia; a) a deceased white-tailed black-cockatoo with evidence of gun shot wound, shown by arrow; b) a deceased white-tailed black-cockatoo found by wildlife officers on the grounds of an orchard. Photographs courtesy of R. Dawson, DEC.

Table 1. A list of the samples collected with case number identification, sample type, DNA identification number and summary of DNA findings for three black-cockatoo forensic cases involving DNA profiling.

Case number	Laboratory case number	Sample type	DNA sample identification	DNA findings
Case 1. Alleged poaching of RTBC nestling from the wild	WGL-BC02	Feathers Eggshell	B08-750 B08-765	Statistical results support the hypothesis that B08-750 was removed from the wild
Case 2. Alleged poaching of three WTBC from the wild	WGL-BC01	Feathers Feathers Feathers	B08-753 B08-754 B08-763	Statistical results support the hypothesis that B08-763 was wild bred and not captive bred
Case 3. Suspected illegal shooting of WTBCs	WGL-BC03	Feathers Feathers Feathers Feathers Feathers Feathers Feathers Feathers Feathers Feathers Feathers Feathers	B09-128 B09-130 B09-131 B09-132 B09-133 B09-134 B09-135 B09-136 B09-137 B09-138 B09-139 B09-140 B09-141	Statistical results support B09-128 and B09-130 to B09-141 were 13 individual white-tailed black-cockatoos

Table 2. Values of average probability of identity (P_{ID}) and random match probability (RMP) calculated using the allele frequencies databases for white- and red-tailed black-cockatoos. Values for white-tailed black-cockatoos are based on 20 loci and 347 individuals, and values for red-tailed black-cockatoos are based on 17 loci and 30 individuals.

Black-cockatoo population database	P_{ID}	P_{IDsibs}	**RMP**	**RMP_{sibs}**
White-tailed black-cockatoos	2.6E-20 (1 in 38 quintillion)	2.4E-8 (1 in 41 million)	9.6E-18 (1 in 104 quadrillion)	8.6E-6 (1 in 116 thousand)
Red-tailed black-cockatoos	1.9E-16 (1 in 5 quintillion)	1.2E-6 (1 in 800 thousand)	6.0E-15 (1 in 166 trillion)	3.7E-5 (1 in 27 thousand)