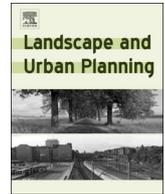




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Research Paper

Genetic sampling identifies canid predators of koalas (*Phascolarctos cinereus*) in peri-urban areasMatthew Gentle^{a,*}, Benjamin L. Allen^{a,b}, Jane Oakey^c, James Speed^a, Lana Harriott^a, Jo Loader^d, Amy Robbins^d, Deidre de Villiers^d, Jon Hanger^d^a Pest Animal Research Centre, Biosecurity Queensland, 203 Tor St, Toowoomba, Queensland 4350, Australia^b University of Southern Queensland, West St, Toowoomba, Queensland 4350, Australia^c Health and Food Sciences Precinct, Biosecurity Queensland, 39 Kessels Rd, Coopers Plains, Queensland 4108, Australia^d Endeavour Veterinary Ecology Pty Ltd, 1695 Pumicestone Rd, Toorbul, Queensland 4510, Australia

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ABSTRACT

An understanding of the threats to threatened species in urban and peri-urban areas is essential to develop successful management approaches. Dog attacks are considered to be a major contributor to koala (*Phascolarctos cinereus*) mortalities in peri-urban areas of north-eastern Australia. Predation could be due to either domestic dogs or wild dogs (dingoes and dingo-domestic dog hybrids), genetically-identifiable groups of *Canis familiaris*. Here, we aimed to use genetic sampling methods to determine or verify the identity, number and successful removal of canid predators of koalas in a peri-urban environment in south-eastern Queensland. Genetic samples were taken from the remains of 12 koalas suspected to have died from predation. Canine genotypes were present on 11 of 12 predated koalas (~92%) and were from wild dogs, not domestic dogs. Most koalas had only one canine genotype identified, suggesting they were killed by a single dog. Our results show that DNA samples collected from deceased prey species can be used to identify the predator, and distinguish between closely-related species, and hybrids of the two. Genetic methods confirmed the identification of the predator obtained through conventional necropsy and support growing evidence that wild dog predation is a significant cause of koala mortality in this region. Strategies to reduce predation on koalas should therefore focus on reducing the impact of free-ranging wild dog populations. This approach is important to identify and target those canids responsible for predation of threatened prey populations, particularly where multiple predators are present and/or predator removals may be controversial.

1. Introduction

The continued development and expansion of urban areas into surrounding habitats poses challenges to fauna conservation around the world. Changes to land use, involving urbanisation, habitat fragmentation, habitat destruction and other processes are recognised as threats to biodiversity conservation (Doxa, Albert, Leriche, & Saatkamp, 2017; Garden, McAlpine, Peterson, Jones, & Possingham, 2006; Gordon, Simondson, White, Moilanen, & Bekessy, 2009; McKinney, 2006). Resident wildlife species must either adapt to the new conditions or be excluded from the altered environment (Allen et al., 2016). Understanding the threats to resident wildlife in urban and peri-urban areas is clearly necessary for wildlife managers and planners to develop

strategic approaches to mitigate these impacts. Understanding threats to wildlife are particularly important given habitat fragments in and around cities are often of high conservation significance, regularly containing rare, threatened and iconic species (Yencken & Wilkinson, 2000).

A common resident in urban areas, the koala (*Phascolarctos cinereus*) is one of the world's most iconic mammals, and a well-recognised Australian endemic species (Goldingay & Dobner, 2014; McAlpine et al., 2015). Populations of this tree-dwelling marsupial in Queensland, New South Wales and the Australian Capital Territory have declined substantially in recent times (McAlpine et al., 2015), and were listed as 'vulnerable' in these areas under the *Environmental Protection and Biodiversity Conservation Act 1999* in 2012. Within the increasingly

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developed area of south-east Queensland, the Koala Coast and Pine Rivers populations have suffered rapid declines (75% in Koala Coast, de Villiers, 2015), with monitoring and modelling studies suggesting local extinctions are possible, even likely (DERM, 2012; GHD, 2008; Rhodes et al., 2011). While destruction of suitable habitat for urban development has been the dominant threatening process (McAlpine et al., 2015), koala populations in these increasingly developed coastal areas remain under additional threat from excessive mortality from disease, vehicle collisions and predation (McAlpine et al., 2015; Rhodes et al., 2011; Thompson, 2006). The decline of critical, coastal source populations also threatens bushland populations, which require immigration to remain viable (de Villiers, 2015; McAlpine et al., 2015). Habitat replacement in these areas is insufficient to prevent further declines (Rhodes et al., 2011) and reductions in mortality factors are also required.

Attacks by domestic dogs can be a significant, unsustainable source of koala mortality (de Villiers, 2015). Dog attacks were responsible for 43% of observed mortality for koalas at Port Stephens, New South Wales (Lunney, Gresser, O'Neill, Matthews, & Rhodes, 2007; Lunney, Gresser, Mahon, & Matthews, 2004). Increasing urbanisation has increased the exposure of koala populations to residential and urban areas, where domestic dog attacks are more likely. Between 2013 and 2016 the Moreton Bay Rail (MBR) infrastructure project, delivered by the Queensland Department of Transport and Main Roads (DTMR), constructed a 12 km rail corridor through bushland and riparian areas north of Brisbane (Petrie to Redcliffe), in south-eastern Queensland (Fig. 1). The rail corridor intersects the Pine Rivers koala population, a significant, but declining, population (de Villiers, 2015). The close proximity of urban areas suggests that roaming domestic dogs may be problematic, but wild dogs (typically dingoes, and dingo-domestic dog hybrids [genetically-identifiable groups of *Canis familiaris* (Jackson et al., 2017)]) are also present in the area (Allen, Goulet, Allen, Lisle, & Leung, 2013; Gentle, Oakey, Speed, Allen, & Allen, 2016; McNeill, Leung, Goulet, Gentle, & Allen, 2016). Wild dogs were known to depredate koalas in the area (Allen et al., 2016), but were an unquantified source of koala mortality. Predation by canids was a significant cause of premature mortality of koalas within close proximity to the rail corridor (Beyer et al., 2018; EVE, 2016). Predation by wild or domestic dogs, or other predators is usually determined from physical evidence at

necropsy and/or characteristics of the kill or kill locations (Fico, Angelucci, & Patumi, 2005; Hanger, de Villiers, Forbes, Nottidge, Beyer, Loader, & Timms, 2017), but it can be difficult to correctly identify the predators using this approach (Caniglia, Fabbri, Mastrogiuseppe, & Randi, 2013), particularly if personnel are not experienced or the carcass is not available or in a suitable condition for necropsy. There is also uncertainty about the relative contribution of wild dogs (dingoes, and dingo-domestic dog hybrids) and domestic dogs to koala predation, especially in close proximity to urban areas. Identification of the main koala predator is required to appropriately direct canid management efforts and assist the recovery of koala populations.

Genetics has previously successfully identified predator species and specific problematic individuals (Banks, Horsup, Wilton, & Taylor, 2003; Harms, Nowak, Carl, & Munoz-Fuentes, 2015). Sampling genetic material, particularly saliva traces, from prey remains has been useful for isolating DNA, with subsequent genotyping contributing to verifying predator identification [e.g. (Caniglia et al., 2013; Marlow et al., 2015; Sundqvist, Ellegren, & Vila, 2008)]. Here, we aimed to use genetic sampling of both predator and prey species to investigate predation on an iconic wildlife species in peri-urban areas, the koala. The primary objective was to (1) construct genetic profiles of canids using microsatellite DNA from saliva traces on koala remains, and compare these to known genetic status groups (Gentle et al., 2016) to verify the identity of the predator/s (i.e. domestic or wild dog [dingo or dingo-hybrid]). The secondary objectives were to 2) use DNA profiling to identify whether single or multiple individual dogs were responsible for koala deaths, and 3) determine whether the individual/s responsible were successfully targeted (i.e. removed) by wild dog control campaigns. Collectively, such information is inherently valuable for managing predation on koalas where multiple predators are present within such an urbanised environment.

2. Methods

2.1. Study site

The study occurred in the northern suburbs of Brisbane, a sub-tropical area on the east coast of Australia (-27.2247, 153.0571). The Moreton Bay Rail Link Koala Tagging and Monitoring Program

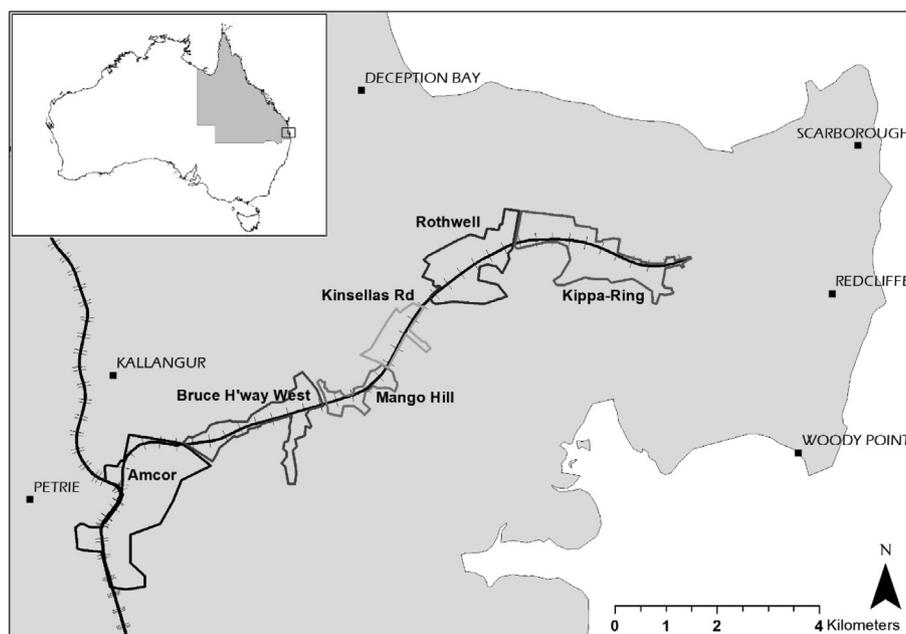


Fig. 1. Location of the six koala monitoring polygons (Amcor, Bruce Highway (H'way) West, Mango Hill, Kinsellas Rd, Rothwell and Kippa-Ring) delineating the rail corridor and adjacent lands, in south-eastern Queensland, Australia. For an satellite image of the area refer to McNeill et al. (2016).

(KTMP2) delineated the 12 km rail corridor and adjacent lands into 6 polygons where koalas were captured, tagged and monitored before, during and after construction of the rail line (Fig. 1). These polygons included koala habitat of varying quality, consisting of intact remnant forest to highly fragmented habitat. The western and eastern extents of the rail corridor contained the most intact bushland habitat and abutted major tidal waterways. Koala densities throughout the rail corridor were relatively high (approx 0.3 koalas ha^{-1}), with predominantly healthy breeding populations compared to other urban areas in South East Queensland (SEQ) (Hanger et al., 2017). However, like other SEQ koala populations, there has been a significant decline in numbers over the last decade or two, resulting primarily from habitat loss and premature mortality from disease, vehicle strike and dog attacks. Wild dogs were found in bushland habitats in northern Brisbane, including within or nearby the rail corridor (McNeill et al., 2016), at an estimated density of approximately 1.5 dogs per km^2 (Allen et al., 2016). Domestic dogs were common in residential, urban areas surrounding the rail corridor.

2.2. Koala samples

From March 2013 until June 2016, koalas within the study area were captured, tagged and intensively monitored as part of the KTMP2 program to manage their health and assess and minimise the impacts to koalas from rail construction and habitat removal (which commenced in 2014) (TMR, 2016). Upon capture, koalas were given standardised veterinary examinations to assess health, and tagged with numbered plastic ear-tags (self-piercing sheep tags, Leader Products, Australia) and fitted with VHF (Very High Frequency, model V2L, Sirtrack, New Zealand) telemetry and/or bio-telemetry (GPS – Global Positioning System, 'K-Tracker', LX Solutions, Australia) tags. Koalas were typically fitted with two telemetry devices (collar and/or anklets) to provide a back-up monitoring system in case of equipment failure or tag drop-off so that the animal could still be located. Upon release, koalas fitted with bio-telemetry collars were remotely monitored by GSM data backhaul to a website. Remote-monitoring allowed 12-hourly GPS location and activity data to be viewed daily. These koalas were visually checked using conventional VHF radio-telemetry every two weeks. Koalas fitted with VHF telemetry devices were only visually checked more frequently at three or four day intervals to facilitate rapid detection of death or ill health. These frequent checks allowed carcasses to be retrieved prior to significant decomposition for diagnostic necropsy examination (DTMR, 2014). In the early stages of the program, before large numbers of koalas were intensively monitored, field personnel would regularly encounter the remains of koalas showing evidence of contact with dogs, suggesting a significant level of predation (EVE, 2013).

Koalas that died during monitoring were necropsied by experienced veterinarians to determine the cause of death. A diagnosis of canid predation as the most plausible cause of death was based primarily upon physical evidence on the carcass identified during necropsy examination (including the distribution of puncture wounds and associated subcutaneous haemorrhage, and muscular trauma), but other physical or circumstantial evidence were also considered (Hanger et al., 2017). Where canid predation was suspected and sufficient tissue, anklet or tag remains were found, samples were collected and stored for genetic analyses. Tissue samples, ear tags, swabs from bio-telemetry collars or Velcro® from tracking anklets were taken from the remains of 12 koalas killed in late 2014/early 2015 (Table 3). Each sample collected was stored separately in a sample jar, either 'dry' or in ethanol (> 70%). Ear tags, tracking anklets and fur were mostly stored 'dry', whilst tissue samples were typically stored in ethanol (Table 1). All samples were stored at room temperature until DNA extraction, which occurred within six months of sample collection.

Dry tracking anklets (Velcro®) and plastic identification ear tags were cut into 2 mm pieces, submerged in Tris-EDTA buffer, shaken and then left overnight to elute any DNA. After addition of a precipitant, the

liquid was centrifuged ($13,000$ rpm \times 5 min) and the pellet was re-suspended in commercial lysis buffer (Qiagen ATL). No pre-preparation of fur and tissue samples were completed, as per manufacturer directions. DNA was extracted from all prepared samples using a commercial DNA extraction kit (Qiagen DNEasy® Blood & Tissue kit).

2.3. Wild dog samples

Control campaigns (primarily trapping) to remove wild dogs were undertaken within the rail corridor and surrounding areas as part of efforts to protect the koala population and to reduce broader negative impacts associated with wild dogs. Where possible, a tissue sample (ear tip) was removed from each wild dog trapped in the broader vicinity of the rail corridor (< 10 km) between September 2012 and July 2015, the period during and after the koala attacks. Tissue samples were stored in ethanol (70–100%) prior to analysis. A universal animal PCR was performed to amplify partial 16S rDNA mitochondrial gene fragment of approximately 700 bp to determine the presence and viability of extracted DNA (Palumbi et al., 1991). Positive control DNA was sourced from wild dog tissue reference samples collected in another study (Gentle et al., 2016; Department of Agriculture and Fisheries, unpublished data) and from a companion domestic animal. DNA was extracted from all samples using the Qiagen DNEasy® Blood & Tissue kit, with overnight lysis, and stored at -18 °C. The negative control utilised was from a DNA-free reaction. Following confirmation of DNA viability, the extracts were tested for the presence of canine DNA with a canine-specific PCR based on hypothetical gene sequence within *Canis lupus familiaris* chromosome4 (Genbank NC.006586.3), observed previously to appear genus specific (J. Oakey, unpublished). Briefly, 2 μL DNA was mixed with 10 μmol each of primers K9F2 (5' AAGTACAGT TCCAGTAGTGC 3') and K9R3 (5' GTCTACCCCAATAGAAAGGG 3'), 5 μg bovine serum albumin, 2.5U MyTaq DNA polymerase (Bioline) and proprietary MyTaq buffering solutions, and water to a total of 25 μL . After initial denaturing at 94 °C for 2 min, the reactions were cycled 40 times at 94 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s followed by a final extension period of 72 °C for 30 s. Amplification was resolved using agarose gel electrophoresis, with canine DNA expected to yield a product of approximately 350 bp and other genera not expected to show any product. Positive controls were the same as the 16S rDNA controls above. DNA extracts testing positive to the presence of canine DNA were further tested using 17 microsatellite loci as described by Stephens (2011) and Wilton (2001). Loci were multiplexed into three amplification reactions and were resolved with capillary electrophoresis using 3500xL genetic analyser. Alleles were determined using Genemarker™ software (Soft Genetics) and results analysed using Genalex® (Peakall & Smouse, 2006).

2.4. Genetic profiles

Samples from koala remains and captured wild dogs suspected of koala predation were analysed to provide a unique DNA profile for an individual, or where present, multiple individuals (koala samples). Recovered canine profiles were tested following the method from Paetkau, Calvert, Stirling, and Strobeck (1995) where dingo, domestic dog and F1 hybrid frequencies were calculated from the frequency probabilities. The approach by Wilton (2001) using Paetkau's method to test hybridisation, with minor modification, was applied to the data. Relative probabilities of allele frequencies were estimated from the purebred allele data for 50:50 (ie. F1) hybrids, and 75:25 or 25:75 (F2) hybrids. Average 3Q [see Elledge, Allen, Carlsson, Wilton, and Leung (2008)] scores were calculated to determine classification.

These data were assessed to determine a) the genotype of the individual canid (dingo, hybrid, domestic dog); b) whether single or multiple canid profiles were extracted from koala samples; and c) whether wild dogs captured during control campaigns were implicated in the 12 koala predation events.

Table 1
Microsatellite results from genetic material samples from deceased koalas identifying the presence of canine DNA.

Koala ID	Sex	Site, Date found dead*	Sample type, storage medium	Microsatellite results
Koala01 ¹	Female	Kippa-Ring, 27/1/15	1. Velcro anklet, dry	One canine genotype
Koala02	Female	Amcor, 7/11/14	1. Neck fur, liquid 2. Liquid from 1 3. Rump fur, liquid 4. Liquid from 3	Four canine genotypes
Koala03 ¹	Male	Kippa-Ring, 29/3/15	1. Velcro anklet, dry 2. Bone, dry 3. Collar, dry	Three canine genotypes
Koala04	Female	Kippa-Ring, 13/02/15	1. Velcro anklet, dry 2. Fur and bone, liquid 3. Liquid from 2	Up to five canine genotypes
Koala05 ¹	Female	Kinsellas Rd, 4/9/14* [#]	1. Velcro anklet, dry	One canine genotype ²
Koala06	Male	Amcor, 17/03/15	1. Unknown tissue, dry	One canine genotype
Koala07	Male	Bruce Highway West, 17/04/15	1. Tissue (skin), dry 2. Fur, dry	One canine genotype
Koala08	Female	Bruce Highway West, 29/01/15	1. Plastic ear tag, dry	One canine genotype
Koala09	Male	Kippa-Ring, 20/4/15	1. Tissue (skin), dry 2. Fur, dry	One canine genotype
Koala10	Male	Amcor, 28/03/15	1. Tissue (skin), dry 2. Swab	Two canine genotypes
Koala11	Female	Amcor, 13/04/15	1. Tissue (skin), dry 2. Fur, dry	One canine genotype
Koala12 ¹	Male	Bruce Highway West, 2/9/14* ^{##}	1. Velcro anklet, dry	No genotypes recovered

*Date of death estimated based on last known activity and location. [#]Tags were recovered 5th February 2015. ^{##}Anklet recovered on 12th February 2015 in Amcor polygon approximately 2 km from last known location.

¹ No carcass remains found, so only suspected wild dog predation.

3. Results

3.1. Koala samples

The remains of all 12 koalas (100%) sampled here were identified at necropsy as predated, or suspected predated by wild dogs. Between one and two samples of, or swabs from, tissue, ear tag, bio-telemetry tag, and/or tracking anklets (see Fig. 2) were taken from the remains of 12 koalas that died in late 2014/early 2015 (Table 1). Extractions were also performed on the transport media (fluids) used to store the samples. Canine genotypes were confirmed in samples from 11 of 12 koalas (~92%) using the canine-specific marker. Ten (10) unique genetic profiles were recovered from all koala samples (Tables 2 and 3), indicating traces of DNA from at least this number of individual dogs from koala remains. Genotypes considered identical were identified from multiple koala samples, indicating that individual dogs were involved in multiple koala attacks. Microsatellite data indicated that between one and five genotypes per locus were recovered per koala (Table 1), indicating that up to five individual dogs (as a minimum) had contact with any one individual koala. Most (7 of 11, ~64%) koalas however, only had one canine genotype identified, suggesting that most were killed by a single dog. Genotypes A, B, C, E and F were found on multiple koalas (Table 2). The genotype recovered from Koala01 was consistent with one of the genotypes recovered from Koala02 and Koala03. Two of the genotypes recovered from Koala04 were consistent with two of the genotypes recovered from Koala02. One genotype was recovered from a single dog attack on Koala06, Koala07 and Koala08. One of the genotypes recovered from Koala03 was also recovered from Koala09 (Table 2). There was evidence of four additional canine genotypes, suggesting up to 14 individual dogs were involved, but results could not be confirmed due to insufficient loci being amplified.

3.2. Wild dog samples

Tissue samples from 39 wild dogs captured during control campaigns conducted within 10 km of the study site were collected for genetic analyses. Microsatellite profiles from these animals were compared to the 10 profiles extracted from the koala samples. DNA profiles

from these wild dogs did not match those from any of the koala samples. These animals were thus eliminated as 'suspects' in the koala deaths. Similarly, positive control DNA from wild dogs sampled within the Moreton Bay Regional Council study area as part of a wild dog hybridisation study undertaken between 2013 and 2016 (n = 251, Gentle et al., 2016 and unpublished data) also failed to provide a match to any of the wild dog DNA recovered from the 11 koala samples. However, given that some additional wild dogs were removed from this area as part of control programs and were not DNA sampled (D. Shiel, Moreton Bay Regional Council, pers. comm. 2018), we cannot discount nor verify the successful removal of the koala attack 'suspects'.

To determine if the koala attacks were committed by wild dogs or domestic dogs, allelic profiles were used to assign each profile extracted from the koala samples to 3Q status (Elledge et al., 2008). Average 3Q classifications indicated that canine DNA from 11 of 11 koalas (100%) were from dingoes or dingo-hybrids, not domestic dogs (Table 3). Most dog profiles (~61%) were classified as 'Probable dingoes', and thus considered as 'dingoes', following Elledge et al. (2008) and Gentle et al. (2016). The remainder were considered dingo-like hybrids, given the dominance of dingo genetics ('Hybrids > 75% Dingo'). Paetkau assignments to either dingo, domestic dog or F1 categories also support the dominance of dingo genetics in allelic profiles extracted from the koala samples (Table 3). No domestic dog profiles were identified, while F1 profiles were extracted from only 2 of 17 canine genotypes. The remainder (15 of 17, ~88%) were classified as 'dingoes'.

4. Discussion

Our results provide demonstrable genetic evidence that free-ranging wild dogs (dingoes and dingo-domestic dog hybrids > 75% dingo), and not domestic dogs, were responsible for killing the koalas we sampled. This genetic evidence supports the findings of the necropsy, where all koalas sampled here were identified as predated, or suspected predated by wild dogs. Verification of the identity of the canid predators of koalas in peri-urban areas is important where multiple canids including domestic dog, dingo and dingo-dog hybrids are present. These methods are also of significant importance, and demonstrate that DNA samples collected from deceased prey species can be used to identify



Plate 1. a–d: a. Koala fitted with plastic identification ear tag, VHF tracking anklet and biotelemetry (GPS) collar. Genetic samples were collected from koalas suspected of dog predation by sampling prey remains and items, including: b. tissues from koala remains; c. identification ear tag and fur; and d. VHF tracking anklet (Source: Endeavour Veterinary Ecology).

Table 2
Individual identity of canine genotypes from microsatellite analysis of genetic material samples from deceased koalas. Genotype identities sharing the same letter indicate a consistent genotype, suggesting shared identities. NA = no canine DNA amplified.

Koala ID	Identity of canine genotype											NA	
	A	B	C	D	E	F	G	H	I	J	Unknown ²		
Koala01 ¹	●												
Koala02	●	●	●							●			
Koala03 ¹	●					●							●
Koala04*		●	●								●		●●
Koala05 ¹				●									
Koala06					●								
Koala07					●								
Koala08					●								
Koala09						●							
Koala10							●						●
Koala11								●					
Koala12 ¹													●

¹No carcass found, so only suspected wild dog predation
²Poor DNA quality resulted in only half the loci being amplified, so confirmation not possible
 * Two unknown canine genotypes

and separate closely-related species, and hybrids of the two. Paetkau assignments and Average 3Q scores for these profiles collectively show a dominance of dingo-genetics in dogs attacking koalas. Profiles extracted from koala remains were verified using both methods to be from dingoes or dingo-like hybrids, as opposed to domestic pets. Correct identification of the canid predator is vital to develop

appropriate management strategies to curb dog predation on koalas. This result demonstrates that, in our study area, efforts to reduce dog predation on koalas need to focus on managing free-ranging wild dog populations. This information is of particular importance for authorities wishing to reduce koala predation in peri-urban areas, where resources are allocated to managing both wild dogs (e.g. trapping) and domestic dogs (e.g. containment) (Gentle et al., 2016). Key threats to koalas in urbanised areas are often assumed to be a result of either disease, vehicle strike or trauma from domestic dog attacks based on significant biases in public reporting of sick, injured and dead koalas (Gonzalez-Astudillo, Allavena, McKinnon, Larkin, & Henning, 2017) and general public unawareness that dingoes are common in peri-urban areas (Allen et al., 2016). Wild dog predation (54% of all deaths) far outweighed domestic dog-related deaths (2%) on the MBR project (Hanger et al., 2017). Strategies to reduce only domestic-dog related mortality would ignore this significant contributor to koala population decline (Beyer et al., 2018).

Determining the identity of the predator is also useful for targeting and verifying removal of those individuals responsible for the impacts. Our analyses of koala samples have identified the DNA of multiple wild dogs on single koala remains and several individual wild dogs on multiple koala remains. Firstly, this suggests that some individual dogs are responsible for multiple koala deaths. Secondly, it is likely that some koalas are being attacked and killed by multiple dogs, possibly working in unison as a ‘pack’, although we cannot discount scavenging may have contributed. Each of these scenarios is likely and supported by previous studies on wild dogs (Banks, Newsome, & Dickman, 2000; Shepherd, 1981; Thomson, 1992). Prey-killing behaviours may be learned, and individual predators may specialise in targeting certain prey groups (Allen & Leung, 2012; Moseby, Peacock, & Read, 2015). Removal of the animals responsible for the predation is required to manage such impacts, and identification of the individuals responsible

Table 3

Paetkau assignment, average 3Q score and assigned status of the 17 wild dog profiles extracted from the samples from deceased koalas. The 10 individual identified genotypes are shown by letters A-J.

Koala ID	Genotype Identity	Paetkau assignments			Average 3Q	
		Ln Lk			Score	Classification
		Dingo	Domestic	F1		
Koala01	A	-2.46937	-15.0401	-4.40649	0.046114	Hybrid > 75% dingo
Koala02	A	-11.8986	-24.0213	-10.5359	0.061201	Probable dingo*
Koala03-velcro	A	-23.4228	-33.0769	-25.3077	0.058111	Probable dingo*
Koala04	B	-18.3665	-22.9321	-13.9361	0.06917	Probable dingo*
Koala04	C	-8.14055	-19.7341	-9.46117	0.064495	Probable dingo*
Koala02	C	-14.5697	-35.4427	-18.4722	0.063802	Probable dingo*
Koala05	D	-11.1983	-29.7601	-14.7042	0.054117	Probable dingo*
Koala06	E	-13.7619	-36.8683	-20.0514	0.047074	Hybrid > 75% dingo
Koala07	E	-16.7768	-39.8668	-23.0707	0.050013	Probable dingo*
Koala08	E	-24.6424	-45.8541	-27.9022	0.061201	Probable dingo*
Koala02	E	-11.8592	-38.3113	-16.4666	0.05804	Probable dingo*
Koala03-bone	F	-12.5823	-18.9896	-16.0847	0.047408	Hybrid > 75% dingo
Koala09-tissue	F	-12.6896	-22.2682	-14.8899	0.058553	Probable dingo*
Koala10-swab	G	-10.1683	-20.0955	-12.0253	0.045975	Hybrid > 75% dingo
Koala11	H	-12.3817	-19.7999	-14.0033	0.058082	Probable dingo*
Koala02	I	-13.0939	N/A	-16.9482	0.104	Dingo
Koala04	J	-13.9783	-45.4812	-15.2448	0.04686	Hybrid > 75% dingo

¹Each entry in the table represents a different profile. For example, the multiple samples from Koala03 identified different dogs. Where identical profiles were observed in more than one sample, they are represented once only in this table. Where individual genotypes are substantially incomplete as a result of poor DNA quality they are listed separately even when the amplified loci have the same alleles.

* Genotypes with an Average 3Q Score between 0.05 and 0.1 were ranked as Dingo by Elledge et al. (2008).

for the damage is the initial step (Banks et al., 2003; Moseby et al., 2015).

While the genetic results support the necropsy findings, our sample size is relatively small (genetic samples extracted from 11/12 koalas sampled) and represents a small proportion (~7%) of the koalas suspected as predated by canids up until June 2016 [n = 159; (EVE, 2016)]. Given that our genetic analyses confirmed the veracity of the necropsy assessments, we can be confident that the other koalas suspected of being killed by wild dogs were in reality killed by wild dogs. Because a large number of koala necropsies performed, rigorous criteria were developed to provide accurate assessments at necropsy. However without this knowledge, attributing mortality to wild dog predation may be overlooked. Difficulties in predator identification from physical signs can occur (Caniglia et al., 2013; Fico et al., 2005; Sundqvist et al., 2008). Genetic testing can assist to objectively and reliably identify the species and individual responsible for the predation (Banks et al., 2003). In our case, we were also able to determine the identity of the predator even in the absence of prey tissues through the genetic sampling of tags or tracking devices.

One limitation to our study is that the quality of the DNA from the koala samples was suboptimal, with none of the extracts providing the entire 17-locus genotype. Suboptimal extractions are likely due to the collection of samples days after death and an extended storage time before analyses. The inferences provided here must be interpreted with DNA quality in mind, because it is possible that genotypes reported to be identical might have been different if all loci were examined, thus the number of individual dogs reported here should be considered a minimum estimate. As such, while it is certainly possible for a wild dog to traverse the 12 km rail corridor, wild dog tracking data collected at the time (see McNeill et al., 2016) indicates that it was unlikely that the same individual could be consistently responsible for the predation of koalas at the eastern and western extent of the site. This conclusion is further supported by information from opportunistic camera trap records and pest control personnel observations that generally identified individual wild dogs in distinct locations. Although koalas were monitored frequently and remains were sampled as soon as practicable by veterinary staff (Hanger et al., 2017), incomplete sampling (e.g. body could not be found) and some degradation of samples had occurred in

some cases. Field sampling of carcasses needs to be conducted as soon as possible (ideally no later than 36 h after the predation event), and samples from multiple prey and the wounds on each prey should be taken to provide sufficient quality DNA for analyses (Caniglia et al., 2013; Fico et al., 2005; Sundqvist et al., 2008).

5. Conclusions

This genetic study will assist in developing target-specific strategies for predation management and also provide feedback on the success of such strategies. Confirming the major role of wild dogs (rather than domestic dogs) in koala deaths at this site will focus management on wild dogs, although there are likely benefits to wildlife from managing all canids in these areas (Doherty et al., 2017; Fleming et al., 2014). The results also suggest that individual wild dogs can be responsible for multiple predation events. Genetic sampling of both predator and prey could be used to both identify and verify the successful removal of the animals responsible for the majority of predation events, rather than reducing population size on a broader scale. For example, a single wild dog (at the western extent of the rail corridor) was likely responsible for half of the koala deaths by predation during the project, as there was no indication from camera monitoring of more than one dog occupying this site and necropsies indicated a distinct killing style (Hanger et al., 2017). Identifying and targeting these individuals is not only important to reduce predation in koala populations, but also for the ethical management of predators, particularly where predator removals may be controversial.

The outcomes of this study add to the growing body of evidence that wild dogs are a substantial contributor to koala mortalities in peri-urban areas (Allen et al., 2016; Beyer et al., 2018; Hanger et al., 2017; Mifsud, 2011). Our work also aligns with broader research identifying wild dogs as key threats to wildlife (Gompper, 2014), and with developments recommending that predator management could be improved by targeting individually responsible predators, and not predator populations or communities generally (Banks et al., 2003; Moseby et al., 2015). Identifying and improving the targeting of predators has global applications particularly in peri-urban and urbanised areas where the value of fragmented habitats for conservation of wildlife species is

increasingly recognised (Chupp, Roder, Battaglia, & Pagels, 2013; Yencken & Wilkinson, 2000). Our approach demonstrates that genetic methods can yield additional valuable information on the impacts, and mitigation of impacts from wild dogs that can help to refine wild dog management.

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