

# Koalas of the Clarke Connors Range



**Final Report: October 2018**

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This final report of the Clarke Connors Range Koala Research Project was undertaken by the Koala Ecology Group between August 2016 and August 2018.

The document has been prepared with the available data collected by the research team. Decisions made by third parties on the basis of this document are solely the responsibility of such parties.

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## Executive Summary

### Koala distribution and genetics-based management units

Koalas were observed across the broad expanse of the Clarke Connors Range between August 2016 and August 2018. Captures were completed at all locations surveyed, from Clarke Creek in the south (-22.63419523, 149.2521391) through to Eungella Dam in the north (-21.139523, 148.365221). Tissue samples from a total of 54 koalas located across the Clarke Connors Range were analysed to determine genetic relationships within this population. Although our analyses have identified two groups of koalas based on genetic dissimilarity, the spatial delineation of these groupings does not suggest the presence of a significant and long-term barrier to gene flow across the Clarke Connors Range at this time, nor the natural separation of the population into more than one geographic unit, on the basis of genetic relatedness.

### Habitat use and ranging of koalas adjacent to the Peak Downs Highway

Ten koalas were fitted with GPS–logging, vhf-transmitting collars as part of this project between August 2016 and August 2017. All collars deployed in the Nebo area for this project have now been recovered. Koala home range size varied considerably, with several dispersals and long-range movements recorded, but also several small ranges of 1 – 3 Ha detected. Unsurprisingly, koala movement patterns also reflected variable nightly moves from as little as 20 m per night to 150 m average movement per night. The directed travel of several koalas appeared to be dispersal, but a long – term study over several years would be required to understand the true nature of the movement behaviour of the koalas in this area. One koala was observed to cross the Peak Downs Highway on numerous occasions, using a creek for access, whilst other koalas were found to remain largely on one or other side of the road.

### Disease testing and results

Of the koalas for which we now have chlamydial test results, only one individual has returned a positive Polymerase Chain Reaction (PCR) outcome, confirming infection. Anecdotal reports suggest that there is a higher prevalence of chlamydial infection in the Nebo area, compared with other parts of the Clarke Connors Range, and our

results lend weight to this theory. Indeed, across both wider surveys we conducted, very little in terms of chlamydial infection or disease signs was evident. The Fauna Rescue Whitsundays Carer group has recorded disease in the Nebo district and beyond, but our surveys revealed a healthy population in general.

#### Koala tree use and diet

Our detailed koala observations by day and night across the Clarke Connors Range allowed us to assess habitat use and determine diet preferences. *Eucalyptus tereticornis* and *E. crebra* were the most preferred species for both day time use and night time feeding, with a range of other species including *E. populnea* being more represented in day time use than feeding observations. South of the Peak Downs Highway and in the Nebo district a preference for the more abundant *E. tereticornis* typical of creek-lines was detected, but surveys of the drier slopes to the north revealed a reliance on *E. crebra* for diet.

## 1.0 Introduction

The Clarke Connors Range is a large, approximately 300 km x 50 km region lying to the west of Mackay, and it is one of the largest wilderness areas in Queensland. The range itself forms the Clarke Connors Range subregion of the Central Queensland Coast Bioregion, adjacent to the Brigalow Belt Bioregion to the west, north and south, and the Sarina to Proserpine Lowlands subregion of the Central Queensland Coast to the east. Feeding into the Proserpine, O'Connell and Pioneer rivers, and holding the headwaters of the Fitzroy and Burdekin Rivers, it has a unique role in the extended ecosystem of central Queensland to the coast and the Great Barrier Reef beyond.

From near Collinsville in the north, south to the Marlborough Hills (some 300 km), this system is also home to an extensive regional koala population. Indeed, the Clarke Connors Range has been identified as a significant refugia within Queensland in the past, and is a potential future koala climate refugia as identified in modelling by Adam-Hoskings *et al.* (2011).

Generally, the koalas are located in the drier woodlands or open forests – mostly on the western aspect of these ranges, although populations still extend to the coast around St Lawrence and Clairview, as well as Sarina. In the latter case, however, these coastal populations are being fragmented as coastal development expands. This regional population has been little studied apart from some preliminary audits near St Lawrence conducted by Melzer and Tucker and the inclusion of samples for the testing of novel genetic markers (Kjeldsen *et al.* 2015). Melzer (pers. comm.) estimates a population of some thousands of koalas to occur in and around St Lawrence. Our work to date has built on the inspections of properties near Nebo (Mt Spencer, adjacent to Mt Adder and Pinnacles) which returned a moderate number of koala sightings, suggesting that the regional population there will also be quite high.

The terrain and geology of these coastal ranges has largely precluded their clearing for agriculture and their development for large-scale resource extraction. In addition, the dominant land uses of the ranges (nature conservation and intermittent cattle grazing) are largely able to coincide with the existing landscape without the need for modification.

However, there are some key threatening processes acting on this landscape. These include inappropriate land management (e.g. fire), increasing climate variability with impacts exacerbated by poor land management, development of the coastal lowlands, and ongoing road and rail kills associated with the Bruce Highway (Clairview, St Lawrence, Waverley Creek) and the Peak Downs Highway (Nebo to Eton), as well as the Hay Point to Coppabella rail link. Critically, there is little known of the full extent, connectivity or ecology of this koala population or of the habitat upon which it is based. Consequently, it is difficult to make conclusions of the significance of any discrete impacts on either the local koala population or on the regional population.

The koala is a medium-sized (4 – 14 kg) arboreal marsupial, that is endemic to Australia and that has become a national faunal icon. Koalas are listed as a vulnerable species throughout Queensland, with habitat loss and degradation major threats to koala populations (Melzer *et al.* 2000). They are a highly specialised folivore, relying almost exclusively on a diet of leaves from *Eucalyptus* and a few other genera. Very little is known about their distribution through the Clarke Connors Range, their patterns of relatedness or health profile or how they use this landscape.

Recently, a contraction in the northern and western extent of the koala's range (Gordon & Hrdina 2005; Seabrook *et al.* 2011) together with widespread declines in abundance across the central and northern parts of its range (McAlpine *et al.* 2015) have prompted national concern for the fate of the species (ECRC 2011), and the Commonwealth listing of the koala as *vulnerable* in New South Wales (NSW), Australian Capital Territory (ACT) and Queensland (Qld) (TSSC 2012). Range contractions are expected to continue with predicted increases in climate variability as well as increased frequency and intensity of extreme weather events impacting on koalas (Adams-Hosking *et al.* 2011), and their habitat. These declines, and predicted climatic changes, have been associated with human-induced increases in greenhouse gasses – especially carbon dioxide, methane and nitrous oxide (IPCC 2013). There are expectations of an increase in maximum and minimum temperatures, increased frequency and duration of heat waves, as well as an increased severity and duration of droughts (Hughes 2003). Consequently, there are widespread concerns about the continued survival of the koala.



Despite the range contractions, koalas have persisted and maintain a widespread, but largely fragmented and patchy distribution across Queensland. Current localities supporting koalas probably reflect refugia from the environmental challenges encountered over the last 25 years. In broad terms these refugia are associated with the mountains, hills and escarpments associated with the coastal ranges (including Kroombit Tops and The Clarke-Connors Range), the precipice sandstones of the Carnarvon and associated ranges (including Blackdown Tableland), and the hills and ranges of the Einasleigh Uplands (McAlpine *et al.* 2015).

Progressive upgrades of the Peak Downs Highway, which bisects this landscape, will contribute to the loss of known koala habitat and reduce its connectivity, and has the potential to a) increase the road kill and b) provide an ecological barrier to koala dispersal and movement. Potential further increases in mining traffic along the Highway resulting from development of the Galilee Basin will exacerbate this existing threat.

The present study was designed to increase knowledge of the Clarke-Connors Range koala population, and facilitate improved conservation management for the species and its habitat. The project includes a consideration of the effective koala management units across the Clarke-Connors Range, and associated ranges as well as investigating koala habitat use and movement patterns in the vicinity of the Nebo – Eton stretch of the Peak Downs Highway.

### 1.1 Koala management units across the Clarke-Connors Range

Investigating and understanding the relatedness of individuals from a species across its distribution has become a key element in devising conservation strategies that reflect environmental and evolutionary processes. One important aim of our work was, therefore, to investigate the distribution of and the genetic relatedness among this population of koalas. This, in time, could allow us to determine whether the koalas along the Clarke-Connors Range are a single, closely related group, or form discrete and separate genetic units. Understanding this can shed light on population connectivity (and hence the significance of any impacts in any one part of the range), and genetic exchange across the area, and hence can inform conservation and management decisions. A finding of several independent, poorly related groups across the region would suggest the existence of historical impediments to gene flow

and barriers to connectivity, or unique colonisation events, which could shape the direction of conservation resource delivery in the long term. By comparison, the existence of a contiguous and genetically related population of koalas across the Clarke Connors Range may lead us to highlight connectivity as a major focus of effective management in the future.

Our goal was therefore to examine the genetic relatedness of koalas across the extent of the Clarke Connors Range, from Clarke Creek in the south to Eungella Dam in the north. Not fully cognisant of the exact spatial distribution of koalas through this region, our initial strategy was to locate and sample any groups of koalas we found at the various localities – perhaps collecting and analysing tissue samples from at least two, and up to four widely distributed sites. However, through repeated surveys we were able to collect samples from the whole region. Our original intention was to supplement the wild – caught koalas with road kill and carer-supplied tissue, however, sufficient samples to undertake a preliminary study for this report were gathered during our surveys. To understand the genetics of this population we compared our data with data for three other sites we were simultaneously sampling – St Bees Island (which is a closed population), Mt Byron in south east Queensland and the Oakey district. This work is ongoing and will contribute to a larger examination of genetic relationships across all of Queensland's koalas.

## 1.2 Koala habitat use, behaviour and movement patterns

Agricultural landscapes include a range of habitats utilised by wildlife, often retained for purposes not directly related to conservation. Other habitats typically found in Australian agricultural landscapes include roadside verges, small patches of uncleared vegetation, riparian vegetation and scattered individual trees - which can all retain elements of value to a range of wildlife species. Quantifying which habitats are available and utilised allows us to prioritise the protection of these habitats, or increase the availability of these habitats within the landscape. Additionally, understanding how or why different habitat features are utilised will provide further insights useful for conservation. Our approach was to examine koala movement through the landscape, especially near roads, to assist in planning to mitigate road impacts associated with the Peak Downs Highway between Eton and Nebo. We chose to do this by utilising GPS logging and VHF radio tracking, to record

movement characteristics (e.g. daily movement distance, and range size) and tree selection. Since koalas are known to increase movement and home range size during the breeding season between September and March (Ellis *et al.* 2009; Ellis *et al.* 2010a), this study was conducted across breeding and non-breeding seasons, to allow us to determine if specific habitat elements are increasingly utilised for movement across the landscape during the breeding season (Matthews *et al.* 2016). Koalas are able to move significant distances in patchy landscapes (White 1999) and the use of habitat corridors is widely accepted as a means of increasing habitat connectivity (e.g. White 1999).

### 1.3 Koala diet in the Clarke Connors Ranges

Although typical food species of the koala are broadly known (Melzer *et al.* 2014), local diet varies - potentially in association with plant leaf chemistry and landscape conditions (e.g. soil nutrients and moisture). To ascertain the regional importance of koala food tree species, we compared daytime and night time tree use to identify species likely to be browse (night time use) and those used for day time roosting (Tucker 2009). Leaf and pellet samples have also collected to facilitate the development of the genetic analysis techniques that are now continuing at Western Sydney University. During the current project we undertook to develop this method ourselves, however, as we present and discuss here, further work is required before it can be used with complete confidence. Nonetheless, we present the results of our development of the method here, as well as the observational method results. Traditionally, diet analysis for koalas have relied on the manual identification and counting of known cuticle fragments in a large number of samples (Hasegawa 1995), but the concurrence between these studies and the observational studies we employed are sufficient (Melzer *et al.* 2014) to engender confidence that we have identified the key diet components within the Clarke Connors Range.

### 1.4 Monitoring Koalas and their habitat

There are multiple approaches to investigating koala habitat use, from indirect faecal pellet counts (Sullivan *et al.* 2004; Callaghan *et al.* 2011) to investigations following koalas using radio-tracking and mark – recapture studies (Ellis 2002; Dique *et al.* 2004).

Over time, the use of indirect methods, such as faecal pellet detection and counts such as the Spot Assessment Method (Phillips & Callaghan 2011), have been found to be unreliable predictors of tree preferences (Matthews *et al.* 2007), and inaccurate estimators of occupancy (Sullivan *et al.* 2002). To ensure a higher level of rigour and confidence in our results, we captured and followed a group of koalas near Nebo, whilst also compiling a database of koala observations across the Clarke Connors Range, both day and night (see diet analysis section, this report). Although this approach is time consuming and involves complex tasks including koala capture, we could generate an index of koala tree use, examine koala movement and also build a study that included disease and health assessments while examining sex ratios and fecundity in the population.

Koala home ranges can indicate the area and number of trees koalas will require, but also allowed us to examine road crossings and the use of non-food trees and compare habitat use between the various tree associations that characterise the local region. We chose to focus on a single location to gather a detailed understanding of the area and to monitor as many koalas as possible given the time and resources available. This approach also allowed us to compare the behaviour of different koalas inhabiting the same landscape, to detect variations due to sex, age or reproductive status. Finally, carrying such studies across seasons was most likely to yield data on changes in species or habitat use in response to climatic conditions (Ellis *et al.* 2010b) so for the current study this was a consideration.

## 2.0 Method

### 2.1 Sampling procedure

#### 2.1.1 Koala capture

Koalas were caught using a standard climb and bag technique (Ellis *et al.* 1995; Ellis *et al.* 2002b; Ellis *et al.* 2011). One team member ascends into the tree housing the koala, and the other members wait quietly on the ground at the base of the tree.



Figure 1. Koala capture: tree ascent.

A plastic bag (40 cm x 20 cm, volume 8 l – a shopping bag) fitted to the end of a telescopic aluminium pole is waved above the koala's head, which generally results in the koala descending the tree to avoid the noise and movement. Once on the ground, the koala is placed in a cloth bag (40 cm x 80 cm, volume 20 l). This is a standard capture method for koalas.

Over the course of the project, we also employed the koala trap method (Hasegawa & Carrick 1995), depending on the terrain and response of koalas to capture procedures: some koalas readily respond to “flagging” and can be captured at the base of trees, other individuals may ignore the rustling bag on the pole and so in some case we deploy our trap. In cases where the koala is in a low overhanging



Figure 2. Koala wearing VHF tracking collar, with young in tree.

branch of the tree, a tarp will be held below the animal and the branch supporting it will be cut, with the koala being caught in the tarp below. To date, no injuries or mishaps to researchers or koalas during capture have occurred.



Figure 3. Koala measurements are carried out on site.

Because the koala is an iconic and charismatic species, it presents opportunities for stakeholder engagement as well as educational opportunities, so our group has been involving the local landholders in searching, tracking and more particularly processing of koalas during this project. As a result, we have been given wide access to properties and gained an insight into the historical perspective of the landholders in regard to the presence and abundance of koalas on their properties.

#### *2.1.2 Koala sampling and collars*

Once caught, koalas are fitted with collar-mounted VHF transmitters and Global Positioning System (GPS) loggers (see image, as used by Ellis (2016)).

Koalas are anaesthetised during sampling procedures, and the animal's age, body mass and gender are recorded. Each koala is physically examined for clinical signs of disease, and for the collection of swab samples and to detect pouch young in females. Swab samples are collected from the left eye, right eye and the urogenital sinus/cloaca (females) or penile urethra (males) for chlamydial disease testing. Two swab samples (6 in total for each animal) are taken from each site to ensure that adequate numbers of chlamydial elementary bodies are dislodged (if the animal was diseased) and collected on the swabs for analysis.

Koala collar shown below has a dorsal GPS unit, AA battery for indication of size, VHF (150 -152 MHz) and proximity logger.



Figure 4. Koala monitoring collar with AAA battery for scale.

## 2.2 Monitoring koalas adjacent to the Peak Downs Highway

The following summaries detail tracking data from koalas with radio collars caught and tracked adjacent to the Peak Downs Highway near Nebo.

### 2.2.1 Zulu: 13201

Zulu was a female adult with a back rider first captured adjacent to the Peak Downs Highway in August 2016. Her back-riding female young (13202) was still with her in November of 2016.



Figure 5 and Figure 6. Koala 13202 and 13201

This koala lives near Denison Creek, utilising the creek system extensively, with occasional excursions away from the creek. Her GPS data reveal 11 movements across the Peak Downs Highway during winter and spring of 2016, concentrated on the watercourse, and we presume that the bridge spanning the creek has provided her with safe passage across the highway.



Figure 7. Tracking map for koala 13201 showing movement across the busy road.





Figure 8. Home range plot for koala 13201

Further analysis of Zulu's movement between November 2016 and April 2017 reveal that this koala has maintained a home range of some 6 hectares, with significant amounts of activity on both sides of the Peak Downs Highway.

The above figure indicates the calculated home range of koala 13201 (Zulu) between November 2016 and March 2017 – breeding season. Note the proximity to Peak Downs Highway, with repeated crossing in the vicinity of the bridge crossing Denison Creek.

Zulu was captured initially on 22 August 2016, re-captured on 4 November and finally recaptured for collar removal on 24 April 2017. Her young (13202) was weaned and left between the November and April captures and in April this female had another young of approximately 3 months in the pouch, which was not removed.

Her condition remained excellent, with body scores of 8, 7.5 and 8 respectively over the course of the study, and no signs of disease were seen for this koala. Swab samples from this koala returned negative results for chlamydial infection. Her mean step length (daily movement distance) was approximately 108 m.

### 2.2.2 Wanda: 13203

Wanda (13203 – also known as Willy-Wanda) was an adult female, captured adjacent to the Peak Downs Highway. Her back young was number 13204, a female that weighed 1 kg at capture (named Vixen-Sierra).



Figure 9 and Figure 10: Koalas 13203 and 13204

Wanda was one of the few koalas to remain relatively settled between visits to the site, ranging among the blue gums (*E. tereticornis*) near the Retreat Hotel rest stop. Her home range area was some 1.5 hectares – a surprising small range in this landscape, and her mean movement distance during the study was about 60 m.



Figure 11. Tracking data for koala 13203

### 2.2.3 Umunga: 13205

Umunga was a healthy adult female with a 1 kg back young (13206 - Tana, male) at first capture. She was captured in August and November 2016 and although sighted again in 2017, had dropped her collar (weak link failure) so was not recaptured. She was observed to be in good condition with a large young separate but in the same

tree, which appeared to be Tana. Her eyes were clear and rump also clear and dry, and since her collar had been retrieved, no effort was made to capture her.

Her tracking data revealed a stable range also within the “Our Retreat” property of some 3.1 hectares and a mean daily move distance of 40 m. This koala was regularly sighted in the blue gums surrounding the motorcycle club facilities near the Peak Downs Highway.



Figure 12. Koala 13205



Figure 13. Koala 13206



Figure 14. Range plot for koala 13205

#### *2.2.4 Silver: 13207*

Silver was an adult female who was caught without a young in August 2016, however she had an elongated teat in her pouch suggesting she had recently weaned a young. Silver was not sighted since first capture, so little is known about her ranging behaviour or tree choices. Given the large movements we have detected for other koalas, she may be several kilometres away from the site of capture.



Figure 15. Koala 13207

### 2.2.5 Muscles: 13213

Koala 13213 was encountered to the west of our main monitoring group near the Peak Downs Highway, so was not collared - but samples were collected. This koala presented with what appeared to be a puncture injury to the right hand, but he was otherwise in good condition. This injury appears to be healing and may be the result of a foreign object or a bite, either from another koala or perhaps from a dog.



Figure 16 and 17. Koala 13213 showing signs of injury.

### 2.2.6 Quinnie: 13209

Quinnie (Koala 13209) was caught adjacent to the Peak Downs highway. She was an adult female who appeared to also have recently weaned her young.



Figure 18. Koala 13209

Quinnie was difficult to locate because she had moved through local properties, eventually establishing herself some 4 km from where we first caught her.

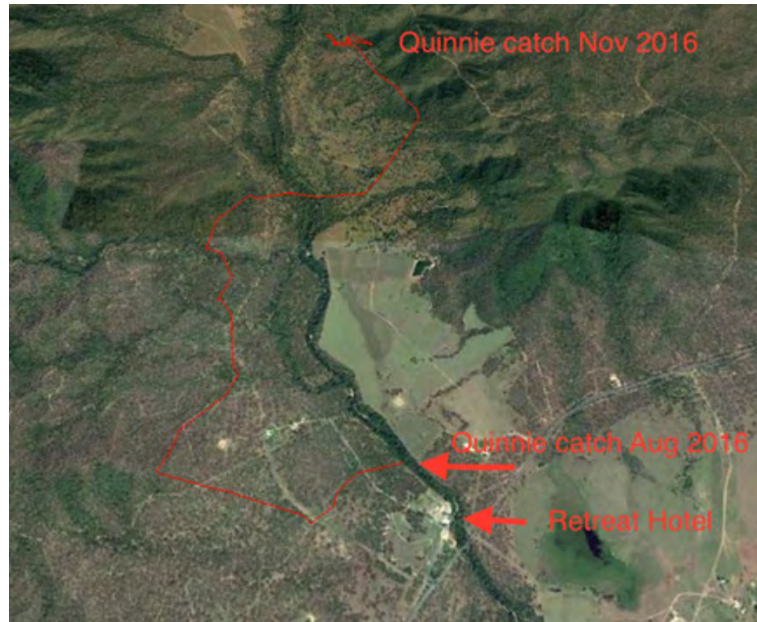


Figure 19. Koala 13209 movement.

#### 2.2.7 Possum: 13210



Figure 20. Koala 13210

Possum (Koala 13210) was captured from an ironbark adjacent to the Peak Downs Highway. Her pouch was empty but she had no signs of ill health.

Between August and November, Possum moved over 10 km to the north, in State forestry land. Her path was relatively direct, indicating movement with a purpose, rather than exploratory wandering. The reasons for her relocating are not known.

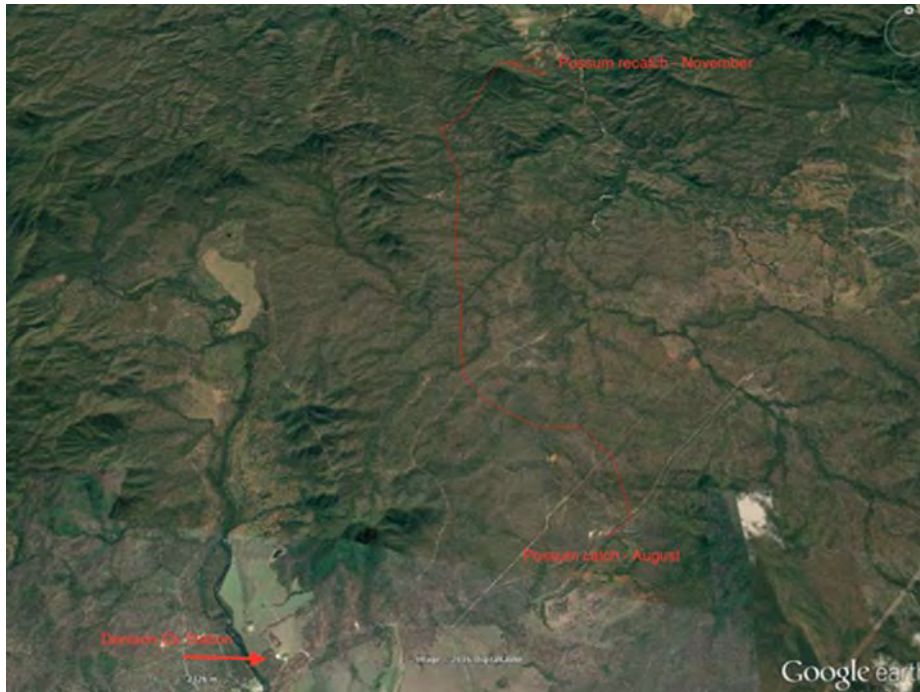


Figure 21. Koala 13210 movement.

#### 2.2.8 Olivia: 13211



Figure 22. Koala 13211

Olivia (13211) was a very young but independent female in excellent condition when first caught in August 2016. When recaptured in November 2016, Olivia had moved across the Peak Downs Highway and onto private property. Her GPS unit had ceased recording, but she had clearly made a large circular pattern of movement, as shown in the track plot below. Olivia had an empty pouch both when first caught and when her collar was subsequently removed, but it is likely that she would now be of

breeding age. Her pattern of movement may be indicative of a juvenile female; these koalas are known to disperse but re-enter their natal range (where they were born) (Tucker 2009) moving through habitat until they settle in an area that they may establish their range in (Tucker *et al.* 2007).

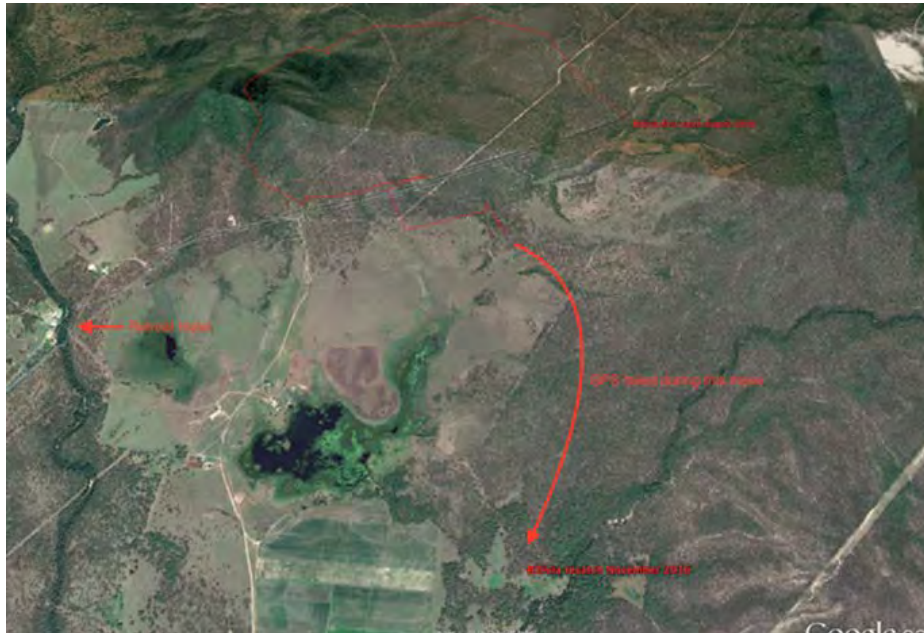


Figure 23. Movement of koala 13211

#### 2.2.9 Neutron: 13212



Figure 24. Koala 13212.



Koala 13212 was an adult male that was caught beside the Peak Downs Highway near Epsom. His sternal gland was active and he had a body condition score of 9 (out of ten) when first caught (8 on recapture). A young adult in very good condition, Neutron was another individual to move in a directed fashion, covering a significant distance including several large hills and valleys (see image of track below).

On both captures (27 August and 9 November) Neutron was located in a pink bloodwood (*Corymbia intermedia*). He grew some 7 mm in head length between captures, but was still some 20 mm shy of expected maximum growth and with only tip wear on his teeth, it is likely that Neutron is “finding his way” as a juvenile adult.

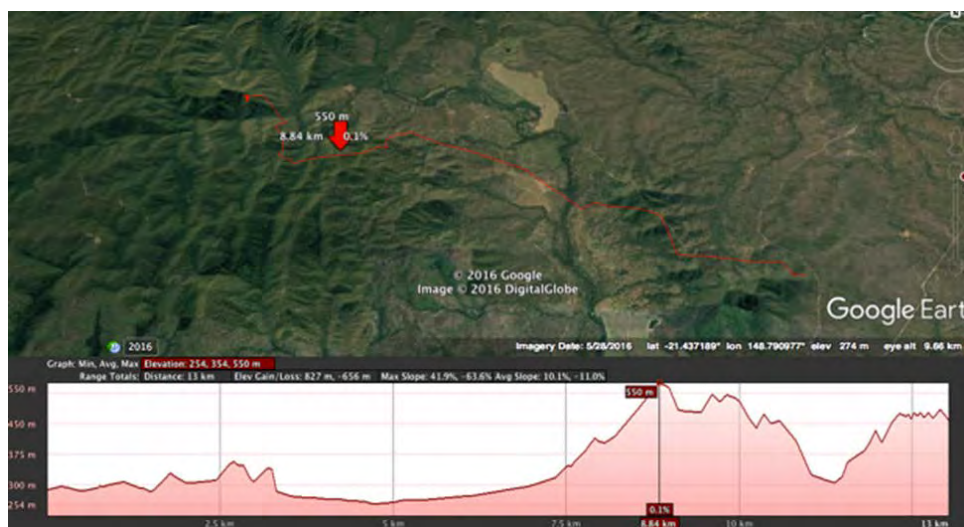


Figure 25. Travel plot for koala 13212

#### 2.2.10 Lola: 13214



Figure 26. Koala 13214 showing signs of disease on rump

Lola was first caught on 31 August 2016, recaptured on 4 November 2016 and finally captured and her collar removed on 24 April 2017. Although first caught in a blue gum (*E. tereticornis*), this koala was commonly sighted in ironbarks (*E. drepanophylla*/*E. crebra*) which dominate her range area in the Epsom State Forest. She has a full ring of wear, indicating age of 6+ years and her body mass and head length have remained constant, despite obvious signs of chlamydial infection – confirmed with PCR analysis.



Figure 27. Koala 13214 showing head.

Lola was one of the koalas that maintained a reasonably consistent range area across the study – in the image below her spring movements are in green whilst her summer range appears in blue. Her range was entirely within the Epsom State Forest to the north of the Peak Downs Highway utilising the open *E. drepanophylla*/*E. crebra* woodland.



Figure 28. Movement plot for koala 13214

*2.2.11 Valerie: 13219*

Koala 13219 “Valerie” was captured during the November 2016 field program and is a healthy adult female.



Figure 29. Koala 13219

Her pattern of movement appears to follow the highway, rarely crossing despite close proximity to the roadside. Comparing her movements with those of Zulu suggests that the Denison Creek crossing utilised by Zulu may not only allow movement across the highway, it might encourage a normal pattern of ranging that follows the creek without exposing that koala to road traffic. Valerie has a considerably larger range size than Zulu (113 Ha compared with 5.8 for Zulu) and her mean travel distance was 82 m, so it is plausible that her range reflects an adaptation to land use that avoids crossing the road.

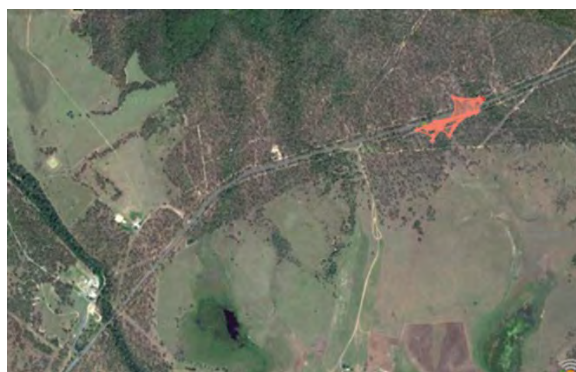


Figure 30. Range plot for koala 13219.

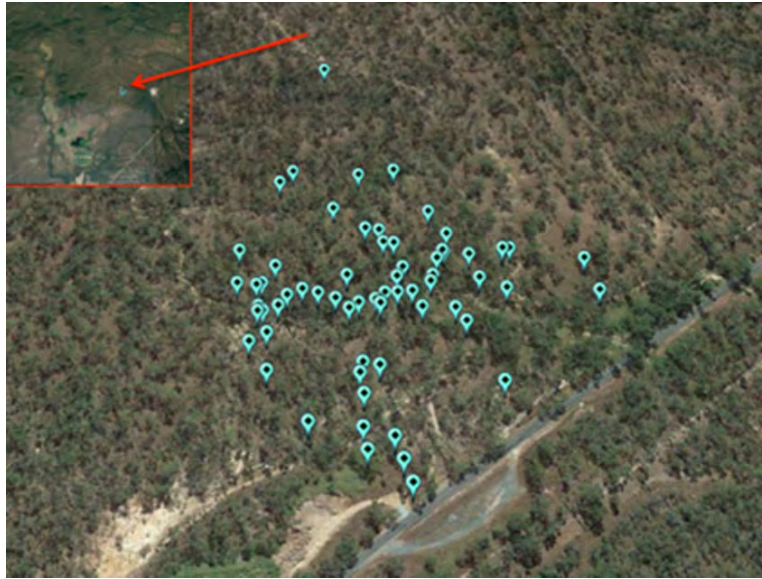


Figure 31. Location data for koala 13219.

### 2.3 Range analysis

Koala home ranges are often used to estimate the amount of habitat each koala requires, to calculate approximate capacity of the landscape and to investigate interactions and resource use by koalas. In previous studies we have detected considerable range shifts between seasons and also interesting patterns of spatial overlap and resource sharing (Ellis *et al.* 2009).

In this study, we found no variation in mean distance of travel per night by specific koalas between seasons, but considerable variation between each koala. For both Spring and Summer periods we recorded between 35 and 80 observations of each koala, discovering movement distances of between 20 m per night up to a mean of 150 m per night. There was some variability between the habitat structure the koalas moved through, including open agricultural landscape, ironbark dominated woodland and the *E. tereticornis* forests near Denison Creek. One koala made multiple movements across the Peak Downs Highway, while a nearby koala (also female) avoided crossing this dangerous road despite having a range that extended beside it.

As a result of the apparent dispersal and large relocations of some individual koalas, range analysis was particularly difficult and, in some cases, impossible. To conduct a minimum convex polygon analysis (MCP) on the movement of koalas such as Possum (13210) and Neutron (13212) who dispersed from the location during our

study would result in range areas of 2246 ha and 896 ha respectively. This compares with seasonal ranges for Umunga (13205) and Zulu (13201) that were in the order of 3.7 ha and 10.3 ha respectively (Summer).

This difficulty can be visualised in Figure 32, which compares the various travel and area use of several of the koalas. Only five of the koalas are shown in this graph to reduce clutter, but it highlights the different movement patterns of the koalas over time, with several making directed movements away from the point of capture, as also displayed in the tracking plots previously shown in this section. For koalas with ranges in the order of 3 – 10 ha which we consider to be standard in these woodlands of central Queensland (compared to the larger ranges of up to 100 ha observed further inland (Ellis *et al.* 2002b)), such plots would record in metres, but here we recorded km, highlighting the distances moved.

Several important conclusions from our study are drawn. Firstly, there appears to be considerable variation in habitat use and ranging behaviour of koalas at this site. Whilst this can be loosely fitted to habitat type, with state forest dominated by *E. drepanophylla*/*E. crebra* considered to offer lower food - resource availability compared to the riparian *E. tereticornis* dominated habitat, koalas within both systems were found to either disperse or remain relatively stable in terms of their range. It appeared that steep slopes, open agricultural country and changing vegetation type provided little barrier to koala movement across this landscape. The extensive relocations (presuming koalas did not eventually return) of several individuals are hard to understand without more detailed population ecology for the area, however it is likely that this is not an unusual behaviour and that koalas do indeed roam widely throughout the landscape.

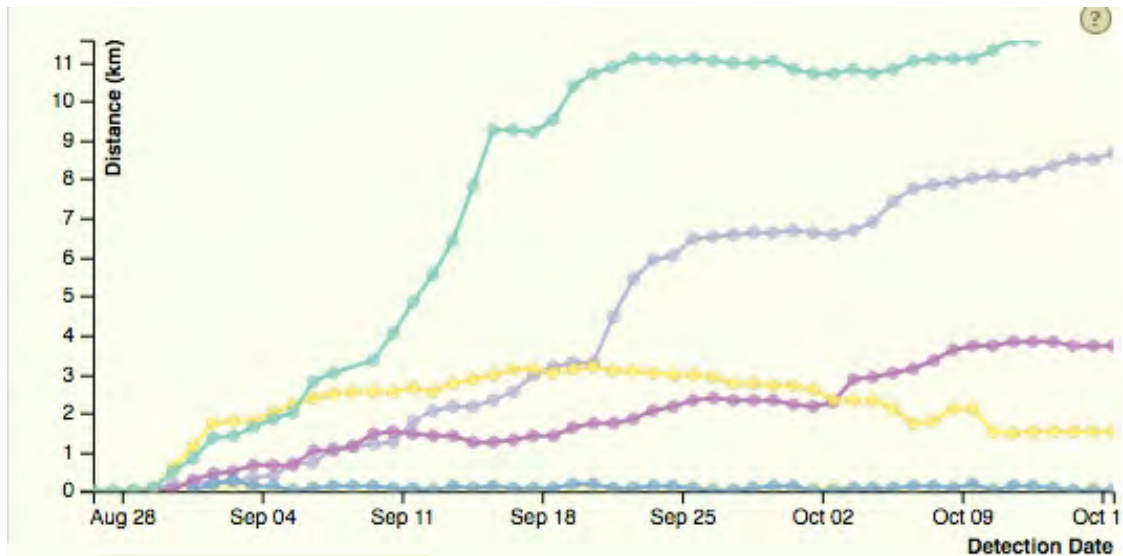


Figure 32. Cumulative distance curves for spring-monitored koalas at the Nebo koala tracking site. X axis intervals are month and date, 2016. Designations: green: 13210 Possum, yellow: 13211 Olivia, grey: 13212 Neutron, purple: 13209 Quinne, and the lower plot dark green: 13214 Lola.

Many other studies have found up to 30% population turnover annually, but with most studies undertaken as part of higher degree projects through universities, the focus remains on the koalas that remain within the logistical boundary of the study. Because we set ourselves no such limit and were able to follow koalas wherever they travelled, we continued to follow koalas that would, presumably, have been lost to less well-resourced studies. One koala in particular, Olivia, presented a huge range, but the somewhat circular movement evident from her GPS log does suggest that this is not a dispersal event, but rather that this may simply be a koala roaming through a very large range. A much longer-term project able to follow koalas further afield, may result in a more complete understanding of koala population dynamics in this region. The assumed relationship between habitat quality and koala home range size has been thrown into some doubt given the vast discrepancies or wide variety of movement patterns of the various koalas in this study.

Secondly, the frequent crossing of the Peak Downs Highway by at least one koala with access to a suitable underpass (over the Denison Creek) highlights that with safe passage, the significant loss of life for wildlife on this road could be reduced. This road is the source of many koala road kills (the subject of a linked but separate study to this report) and has the potential to be both a barrier to gene flow in the

district and a significant driver of koala population decline across its length. Upgrades to this road and the adjacent fencing of private properties that are sensitive to wildlife passage, and mitigation efforts that limit access of wildlife onto the Peak Downs Highway are important steps to secure the future of this population.

Our surveys, tracking and monitoring during this project also highlight the value and consideration of private landholders and their role in the future of this population. All of our koala tracking was undertaken either on private properties or in state forests, many of which had concurrent grazing lease arrangements. Hence, landholder efforts in pest and fuel load management and their retention of koala habitat is a key input into koala population security in the area in the long term.

Finally, our frequent interactions with local people as we conducted our work indicated that these people consider the population of koalas in the Nebo area to have increased in the last 30 to 40 years. Whether this is an accurate reflection of koala population dynamics remains unknown, but our study provides a benchmark against which future work can measure such assumptions.

### 3.0 Koala tree use and diet analysis

Studying the tree choices of koalas by day and night can be an effective way to understand the diet choices they make, because they tend to eat primarily (but not exclusively) at night (Melzer 1994; Tucker 2009). There are other methods in use, some of which have been found to be simple but inaccurate (such as the Spot Assessment Technique (Matthews *et al.* 2007; Phillips & Callaghan 2011) and others which are highly accurate but resource intensive (Hasegawa 1995; Ellis *et al.* 1999). At present, there is significant effort directed toward developing a genetics-based approach to diet analysis in the koala, with a recent Queensland Government grant to the University of Western Sydney for a project titled: “Novel characterisation of koala diets and habitat quality using faecal molecular analysis”, which we hope will complete the work we have started here. We have previously used both direct observations and faecal cuticle identification to determine koala diets, and are currently collaborating with the Western Sydney University group investigating the future of genetic analysis of faecal material to predict diet, but for the present study, we have relied primarily on observation of koala tree choices to guide our assessments. The genetic results we present here indicate that this method will be

viable in the future, and do correlate closely with our observational data. Pellets and leaves have been collected and are stored for a longer term assessment in the future, but because our previous work on koala diets across Queensland has revealed a close association between night time tree use and diet preferences (Tucker 2009; Woosnam-Merchez *et al.* 2012; Ellis *et al.* 2013), we have relied heavily on this approach to generate an understanding of koala diets within the Clarke Connors Range for this report.

### 3.1 Methods

#### 3.1.1 Observational assessment

We monitored koalas closely in the Nebo district by using radio tracking, as discussed previously in this report. In addition, we conducted wider surveys across the Clarke Connors Range, to collect samples for genetic analysis and disease testing, which are also discussed elsewhere in this report. During both of those activities we conducted independent day and night time surveys of koalas, to provide information on koala tree choices.

Daytime surveys were conducted on foot at a local area level. Repeated daytime surveys of individual properties adjacent to the Peak Downs Highway and the adjacent state forests were combined with our road – side and associated vegetation surveys conducted during the wide surveys of August 2017 and 2018 (see distribution and genetic analyses). In all cases survey sites were selected based on access and an “educated guess” regarding the suitability of the landscape (generally after sightings of koalas were made). Though hence subjective, this allowed us to target the “most likely” habitat, based on our years of experience in this area of Queensland, and resulted in a high rate of koala detection. As is noted in the section on koala distribution, koalas are widely spread across the Clarke Connors Range and failure to detect koalas was indeed the exception in surveys.

To complete each survey, three team members each walked in a different direction away from the survey central point, making an approximately 20 min out and 10 min return trip along the same bearing (e.g. 180° return). This allowed a more intensive first search transect with a confirmation or checking return transect, conducted by the single observer, repeated in three directions but comprising a compact and easily repeatable koala detection survey in almost any landscape. The use of the central



GPS point was key to ensuring that the day time surveys were able to be repeated at night time, whether in the same landscape or at any new, unfamiliar location.

In addition, koalas were regularly spotted from our vehicle, either during daytime driving or during nocturnal vehicular spotlighting surveys. At locations with well-maintained vehicular access tracks (such as some private landholdings where we were granted access) we used nocturnal, in-vehicle spotlighting surveys.

### *3.1.2 Genetic analysis of leaf and faeces*

Small fragments of DNA remain intact in faeces and can be used to determine diet species in herbivores or in the case of carnivores, prey species. To do this for koalas, faeces are collected, DNA is extracted and a small fragment of DNA is amplified using suitable primers. Next gen sequencing is then used to generate a large number of copies of each fragment, which is in turn used to determine number and approximate proportion of each Eucalypt species found in the sample.

Typical diet species for koalas are known (Melzer *et al.* 2014), which allowed us to generate a reference database of the most relevant species for the Clarke Connors Range. Leaves from three specimen trees of each species were collected with two to five fresh leaves placed in a centrifuge tube filled with self-indicating silica beads (chem-supply 2.0-5.0 mm/4-10 mesh) to dehydrate. Samples were stored at ambient room temperature until DNA extraction. Faecal samples were stored in the same way.

As DNA fragments in faeces are themselves typically fragmented, target regions must be short and variable, and flanked by conserved genomic regions to allow solid primer designs. Selecting these target regions allowed reliable amplification of a region where fixed, species-specific variations exist. Literature searches revealed several possible chloroplast regions that have been used for species identification for diet analysis in herbivores, for example the trnL region (Valentini *et al.* 2009). This gene contains a small (36bp) P6 loop region which was found to be suitable for diet analysis in a number of small herbivores (Soininen *et al.* 2009). However, the single genus diet of koalas has certainly proved more challenging for us. Alignment of this region in publicly available eucalypt chloroplast genomes, indicated that this region was not variable enough to distinguish at the species level. Based on this finding, a more eucalypt-DNA specific search was conducted.

A thorough analysis of the chloroplast genome Australian *Myrtacea* revealed several hypervariable regions, including the intragenic spacers between the rpl 2 – tRNA – His and psbA (Bayly *et al.* 2013). Based on this finding, sample sequences spanning this region, from *E. microcorys*, *E. calmadulensis*, *E. elata*, *E. grandis*, *E. microcorys*, *E. obliqua* and *E. umbra* were downloaded from the National Center for Biotechnology Information (NCBI) webpage and aligned manually using BioEdit. Both intragenic regions were considered suitably variable for the purpose of species identification. A set of already published primers (Goulding *et al.* 1996; Vaillancourt & Jackson 2000) to amplify these two regions were used.

#### DNA extraction and amplification

Approximately 5mg of dry leaf was placed in a 1.5 ml microfuge snap lock tube along with 3 (xul) and 10 (xul) metal beads and placed in a bead beater for 5 minutes (or until ground to a fine powder). DNA was extracted using the Qiagen DNEasy plant mini kit according to the manufacturer's instructions resulting in good quality, 10-20 ng DNA/ $\mu$ l extractions. DNA quality and quantity was verified on a 1% agarose gel.



Figure 33. Gel image of DNA extraction

A polymerase chain reaction (PCR) was conducted using the trnH primer as forward primer and rpl2 or psbA as reverse primer. The reactions were conducted as 20  $\mu$ l reactions consisting of the Qiagen Taq PCR core kit with the following concentrations: 2  $\mu$ l 10x Taq PCR buffer, 200  $\mu$ M of each dNTP, 10  $\mu$ M of each primer, 0.5 U Taq polymerase and approximately 5ng DNA. Comparisons with and

without the use of the Q solution showed better results without. Thermal cycling conditions were set with a 5 min denaturation step at 95°C followed by 35 cycles of 1 min at each of the following: 94°C, 48°C, 72°C and a final extension of 5 min at 72°C.

#### Sequence amplification and alignments

Based on the resulting gel image, the trnH-rpl2 amplicon was selected for Sanger sequencing. Approximately 200 ng of DNA along with the trnH and psbA primers were sent to the Australian Genome Research Facility (AGRF) for sequencing.

Figure 34 shows quality, single copy amplification of an approximately 150pb long trnL – psbA intra-genetic region (circled in green). Amplification of the rpl2 – trnH region created un-specific amplification (circled in red).



Figure 34. Gel image of PCR reactions

Resulting sequences were imported into BioEdit, aligned manually, and showed six clusters of identifiable units. *E. grandis*, *E. microcorys* and *Lophostemon confertus* can be identified to species level while the two *Corymbia* species, the two Ironbarks (*E. melanophloia* and *E. crebra*), and *E. major* and *E. tereticornis* form three distinct pairs, where the pair cannot be told apart from each other, but have a unique sequence compared to the other five units.

## 3.2 Results

### 3.2.1 Observations

Our koala-spotting survey database for this project now includes 134 koala observations in trees identified to species level (other observations were used for location data only). During day time, koalas were observed in ironbark (*E. crebra* or *E. drepanophylla*) on 41% of observations, and blue gum (*E. tereticornis*) on 30% of observations. Other species made up the balance of observations with bloodwoods the highest representation at almost 6% (Table 1). By comparison, night time observations indicated a clear preference for *E. tereticornis* (56%), with ironbark (*E. drepanophylla*/*E. crebra* 39%) being the only other species with significant representation. As a result of this preference for *E. tereticornis* at night, overall proportional use of *E. tereticornis* and *E. drepanophylla*/*E. crebra* was relatively similar (37% and 40% respectively) with koalas spending some 23 % of their time in a range of other species including bloodwoods and *acacia* species.

Table 1. Tree use by koalas in the Clarke Connors Range

Species / Type	Observations	Day	Day %	Night	Night %	Overall %
<i>E. drepanophylla</i> / <i>E. crebra</i>	54	40	40.0	14	38.9	40.3
<i>E. tereticornis</i>	49	29	29.6	20	55.6	36.6
<i>E. populnea</i>	3	2	2.0	1	2.8	2.2
<i>Corymbia intermedia</i>	2	2	2.0	0	0	1.5
Other <i>Corymbia</i> spp.	6	6	6.0	0	0	4.5
Other	20	19	19.4	1	2.8	14.9
Total	134	98	100	36	100	100

### 3.2.2 Genetic analysis

The eucalypt samples were sequenced using AGRF Sanger sequencing under the contract CAGRF10259 and CAGRF11705. Samples under these contracts were used to create the reference library.

Raw chromatograms were parsed from ab1 file format to fastq. The fastq files were stitched by aligning the forward and reverse reads using PEAR1 (version 0.9.5). For each sample all the replicates were denovo assembled using Geneious2 (version 6.1.8). The consensus sequences from the denovo assemblies are now considered as the reference for each of the species.

### 3.2.3 Analysis of collected faecal samples

MiSeq koala faecal sample DNA paired-ends reads were assembled by aligning the forward and reverse reads using PEAR1 (version 0.9.5). Primers were identified and trimmed. Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8) 3 USEARCH4 (version 8.0.1623) and UPARSE software (Edgar 2013). Sequences were quality filtered using usearch tools and to obtain the number of reads mapping to a species in the reference library, filtered reads were mapped to the *Eucalyptus* reference library with a minimum identity of 90%.

Relative abundance for each operational taxonomic unit (OTU) was provided by the Australian Genome Research Facility (AGRF) in an excel format as indicated below in Table 2. Each column corresponds to the samples (faecal pellet) and each row corresponds to the number of times a sample appears in a particular reference. The following table shows results for ten samples. *E. crebra* is prolific in these results, however the capacity to distinguish between species remains subject to caution at this stage and the high number of non-matches indicates that we have not developed this technique sufficiently to detect diet preferences with sufficient confidence.

Table 2. Matching faecal DNA to reference sample DNA for Clarke Connors koalas.

OTU ID	CQ-1-a	CQ-1-b	CQ-1-c	CQ-1-d	CQ-1-e	CQ-1-f	CQ-2-a	CQ-2-b	CQ-2-c	CQ-2-d
<i>E-crebra_</i>	230697	236870	144221	201271	296343	249614	97571	164455	113925	174903
<i>E-populnea</i>	1682	1664	927	1392	2197	1912	283	439	305	542
<i>C.-macculata</i>	5	5	4	5	12	4	3	1	3	7
<i>C.-tessellaris</i>	3	3	3	5	17	8	1	0	2	1
<i>E-major</i>	7	1	2	4	7	3	1	3	2	0
<i>E-grandis</i>	1	2	0	2	8	5	6	0	0	0
<i>L-confertus</i>	0	1	0	2	2	0	0	0	0	0
<i>E-microcorys</i>	0	0	1	0	2	3	0	1	0	1
<i>E-tereticornis</i>	0	0	0	0	2	0	0	0	0	0
<i>E-exserta</i>	0	0	0	0	0	0	22	0	0	0
no-matches	20462	9077	4733	7291	5747	7663	6373	29909	19696	8185

To highlight the concern we have with these results – and for their ability to accurately identify all diet components for koala, Figure 35 plots the ratio of known to unmatched eucalypt DNA in a single koala faecal sample.

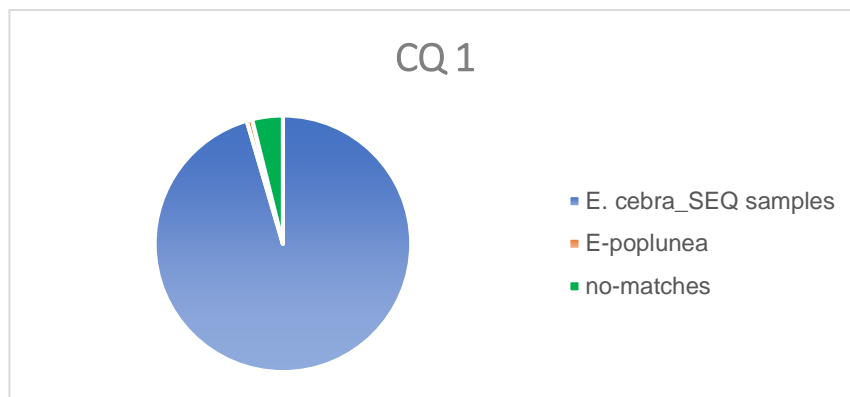


Figure 35. Unmatched to matched DNA ratio in a koala faecal sample (randomly selected). Note: the match to *E. crebra* from south-east Queensland, minor match with *E. populnea* and large proportion of non-matched DNA.

### 3.3 Discussion

Our observations suggest that koalas prefer *E. tereticornis* for diet, with *E. drepanophylla* / *E. crebra* also being utilised at night, and hence also a probable diet species in the Clarke Connors Range. No other species were observed to be used by koalas at night in this study to date. Koalas were observed using bloodwoods (*C. intermedia* and *C. erythrophloia*) during day time, as well as being observed in *E. orgadophila*, *C. tessellaris*, *E. populnea* and a range of other species in low numbers.

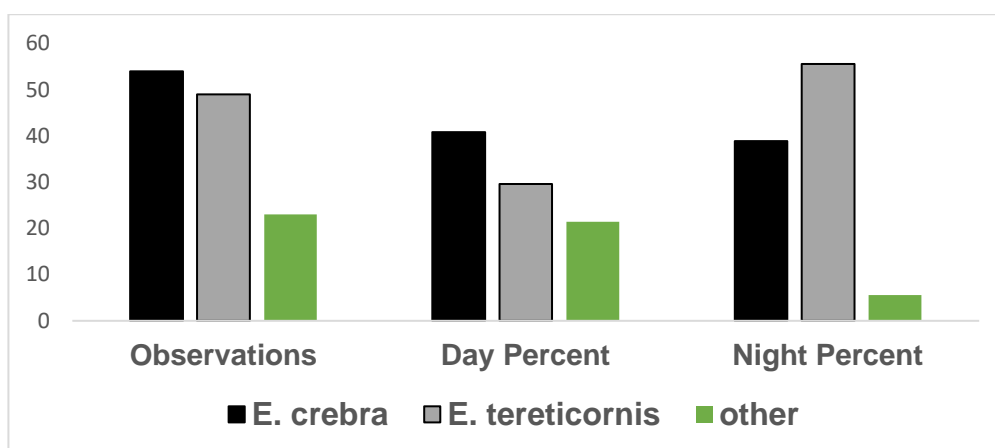


Figure 36. Koala tree use by day and night in the Clarke Connors Range

We were somewhat surprised at the low occurrence of night-time use of *E. populnea* during our study, considering that in a previous work in central Queensland this species was one of the highest for occurrence in diet (~60%) (Ellis *et al.* 2002b). However, the tree associations characterizing the slopes and valleys of the Clarke Connors Range were not dominated by *E. populnea* (despite it being well represented) and spatial variability in diet choice is not unexpected.

The use of non-food species by koalas in the Clarke Connors Range was not as extensive or exclusive as reported in other areas of Queensland (Ellis *et al.* 2002b), but our field study was also not through the height of summer, during which koalas are known to seek refuge in whatever shady trees are available (Ellis *et al.* 2010b). The extensive use of non-food trees by koalas has adaptive significance in hot periods, but must also reflect vegetation availability. The benefits to individuals of leaving food trees to shelter in non-food species during the day must outweigh the energetic costs of such behavior, which in open landscapes such as were surveyed in this study, would be extensive. It has been reported that the tree species selected for day time use in hot environments will be characterised by denser canopies than the food tree species (Pfeiffer *et al.* 2005) so there is likely to be a physiological benefit of shade in warmer weather (Ellis 2010) or prey avoidance advantage (Melzer *et al.* 2003). Our conclusion is therefore that we were able to identify the diet species preferred by koalas in the Clarke Connors Range, but may find a significantly greater reliance for day time roosting on more-dense, non-food species in summer in this region.

There are several approaches currently being developed to identify DNA fragments from eucalypt in koala faeces and it is very likely that within the next few years this method will prove to be the most efficient approach to analyzing koala diet preferences. Our results indicate that we can detect some, but definitely not all, the components of koala diet, and even though our DNA results appear to confirm the observation results (*E. crebra* being highly selected), the high rate of unmatched samples means that this method is currently inappropriate to build conclusions about koala diets for the Clarke Connors Range.

#### 4.0 Koala population health in the Clarke Connors Range.

*Chlamydia pecorum* and *C. pneumoniae* are known infectious agents of the koala, with *C. pecorum* being the more pathogenic of the two species (Jackson *et al.* 1999a; Devereaux *et al.* 2003a). Surveys of wild koala populations in several Australian states have also revealed highly variable infection rates ranging from 0% up to 100% for *C. pecorum* and 0% to 53% for *C. pneumoniae*. (Brown *et al.* 1987; Polkinghorne *et al.* 2013; Patterson *et al.* 2015). *Chlamydia pecorum* infects the ocular, urinary and genital mucosae leading to blindness, cystitis and infertility, respectively (Blanshard & Bodley 2008; Wan *et al.* 2011) but infections by *C. pecorum* often present with no clinical signs, especially in the reproductive tract, and therefore go unnoticed during population surveys based solely on observations (Polkinghorne *et al.* 2013). Together, chlamydial infections contribute not only to the overall health decline of koala populations, but also to reduced reproductive rate (Obendorf 1981; Canfield 1989; Higgins *et al.* 2005).

In addition to *Chlamydia*, a number of other bacterial organisms including *Mycoplasma* and *Ureaplasma* may contribute to population decline, but their epidemiology and pathogenicity have yet to be fully characterised in the koala. In humans and many animal species, *Mycoplasma* and *Ureaplasma* have been responsible for lesions similar to those of chlamydiosis, including expression of clinical disease in urogenital, ocular and respiratory infections (Waites & Taylor--Robinson 2015). The prevalence and pathogenicity of these two bacteria in koala populations is currently unknown.

In contrast, *Bordetella bronchiseptica* infections have long been documented in captive koala populations (Canfield *et al.* 1986). In the koala, *B. bronchiseptica* respiratory infections exhibit similar clinical signs to those of *C. pneumoniae*, such as mucopurulent nasal discharge, sneezing and/or coughing (Blanshard & Bodley 2008). Respiratory disease caused by *B. bronchiseptica* infection is extremely serious and severe and has been known to affect individual animals as well as groups; if left untreated, infection results in rapid mortality (Blanshard & Bodley 2008).



#### 4.0.1 Collection of samples and assessment of disease

The health of koalas was investigated during captures undertaken for the habitat use work using radio collars, and the broader surveys across the Clarke Connors range.

All koalas underwent a simple health assessment including the collection of swabs to detect chlamydial infection. The samples were collected using sterile cotton-tipped aluminium or plastic shafted swabs (Copan; Interpath Services, Melbourne), with vigorous swabbing around the inside of the koalas' eyelids (conjunctiva) and by inserting a swab 3 cm inside the urogenital sinus or 2 cm inside the penile urethra, to collect elementary bodies or dislodge organisms (Weigler *et al.* 1988; Vogelnest & Woods 2008). Swabs were stored dry at -20°C for later analysis.

This assessment is routinely conducted in conjunction with photographs taken of each koala's eyes and rump, to document any signs of clinical disease such as keratoconjunctivitis at the ocular sites, and/or cystitis in the urogenital tract. Clinical signs of ocular infection can include reddening and/or swelling of the conjunctivae, serous discharge and partial or complete closing of the eye(s). Severe cases can cause reddening and granular tissue to form on the conjunctiva and a purulent discharge to exude from the eye(s) resulting in staining or loss of fur around the eye. Corneal opacity may also be present. Clinical signs of urogenital infection can be distinguished by brown/yellow staining of the fur on the rump of the animal. The rump may be wet from continuous soiling due to incontinence and in severe cases scalding and ulceration of the rump may occur.

The koala body condition index from Ellis and Carrick (1992) is used to assess the amount of muscle tissue around the scapula as an indication of any deterioration in the koala's health. This is assessed on a scale of 1-10, with 10 being excellent muscle/fat condition and anything below 5 being very poor.

A variety of methods and approaches have been used to detect and analyse chlamydial infection in koalas over time, including cell culture (White & Timms 1994) serological techniques complement fixation test (Weigler *et al.* 1988; White & Timms 1994), Clearview test (Hanger *et al.* 2013) and visual observations for clinical signs of disease (Cockram & Jackson 1981; Mitchell *et al.* 1988; White & Timms 1994). These methods are largely considered to be unsuitable for detection of chlamydial infection due to their low sensitivity (when detecting chlamydial elementary bodies)

and high false-negative rates (visual observations)(Ellis *et al.* 1993; Jackson *et al.* 1999b). Cell culture, while considered sensitive, is labour and resource-intensive and requires viable bacterial samples, which are difficult to maintain when sampling in remote locations (White & Timms 1994). A number of serological tests including the compliment-fixation test, used sheep and guinea pig antigen tests which provided a high rate of false-negative results (White & Timms 1994). Molecular methods involving PCR are considered the most reliable for providing accurate data of the prevalence of chlamydial infection in free-ranging koala populations (Jackson *et al.* 1999b; Devereaux *et al.* 2003b). As a result, we used standard PCR (using gel electrophoresis, described below) to detect koala *Chlamydia* from our samples.

#### 4.1 Method

##### 4.1.1 Multiplex Primers and dual-labelled fluorogenic probes

Nucleotide sequences for the multiplex rtPCR primers and hydrolysis dually fluorophore-labelled probes were designed using Beacon Designer 8.2 (Premier Biosoft International, Ltd., Palo Alto, CA) based on sequence data available from the GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) database. Commercial sources provided the primers (GeneWorks, Adelaide, SA, Australia) and the dual-labelled probes (Biosearch Technologies, Novato, CA, United States) used in this study. The dual-labelled probes were prepared by labelling reporter dyes to the 5'-terminus and Black Hole quencher dyes to the 3'-terminus of synthesised oligonucleotides. Primer sequences included in each multiplex were compared using the basic local alignment search tool, BLAST-n (<https://www.ncbi.nlm.nih.gov/BLAST/>).

##### 4.1.2 Multiplex real-time PCR

We used two multiplex rtPCR panels and each sample was assayed with both multiplex panels. Multiplex panel 1 included the housekeeping gene, koala beta-actin, in order to confirm the presence of host cells in the sample and as a control for DNA quality. As well, multiplex 1 included universal primers targeting conserved regions of the relevant genome for the detection of isolates of *Chlamydia*, *Mycoplasma* and *Ureaplasma*. Multiplex 2 PCR panel was designed to be run subsequent to multiplex 1 to avoid the need for repeat screening of the endogenous control gene. Multiplex 2 served to validate *Chlamydia* results from multiplex 1, by identifying the known koala-infecting *Chlamydia* species, *C. pecorum* and *C. pneumoniae*, and also to detect *B. bronchiseptica*.

#### 4.1.3 DNA amplification

Both multiplex rtPCR panels were carried out independently with a total reaction volume of 25 µL per multiplex assay, containing 10 µL of mastermix (SensiFAST™ Probe No-ROX kit, Bioline, London, UK), 0.4 µM of each primer and 0.2 µM of probe, 8.2 µL of sterile distilled water in multiplex 1 and 8.65 µL in multiplex 2, and 5 µL volume of DNA template was added to each reaction.

Cycling parameters for the multiplex rtPCR panels were as follows: initial denaturation at 95°C for 5 min, and then 38 cycles at 95°C for 5 s and 10 s at either 57°C (for multiplex 1) or 58°C (for multiplex 2), both run on a Rotor-Gene Q™ (QIAGEN, Doncaster, VIC, Australia). Fluorescent data were acquired during the annealing/extension phase. DNA extracted from the reference strains/isolates were used as the relevant positive control. A no-template control sample containing water instead of DNA template was included in every reaction run.

A standard curve for the *C. pecorum* PCR assay in Multiplex 2 was established with a view to quantitating bacterial load in a sample. The *C. pecorum* quantitative PCR (qPCR) assay was calibrated using a known standard of quantitated cell culture derived semi-purified koala *C. pecorum* genomic DNA diluted to  $10^7$  -  $10^1$ , as determined by spectrophotometry using the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, VIC, Australia), and run in triplicate to determine the detection limit of chlamydial copy number.

#### 4.1.4 Validation of multiplex real-time PCR panels

Validation of both multiplex PCR panels using reference strains/isolates to establish accuracy, followed published guidelines (Raymaekers *et al.* 2009) whereby specificity of all oligonucleotide sequences was verified using BLAST-n and each individual target was assayed in triplicate as a singleplex using SensiFAST SYBR No-ROX and SensiFAST Probe No-ROX (Bioline), using the reference strain for each assay to establish sensitivity. Melt-curve analysis of the SYBR assay was used for specificity.

## 4.2 Results

Of the koalas that we have collected samples from for this element of the study, only one has returned a positive swab. This female, Lola (13214), is a female residing in Epon State Forest near Nebo, who was last sighted in August 2017. Despite this, the Fauna Rescue Whitsundays Carer group reports that a significant proportion of

the koalas they receive from the Nebo area are presented as a result of chlamydial disease.

As indicated in Table 3, no signs of disease were apparent during our broad-scale surveys, and only the one (see above) swab sample returned a positive PCR result.

Table 3. Koala observation records and chlamydial screening results for koalas in the Clarke Connors Range koala research project.

UQ #	Name	Date	Left eye	Right eye	Rump colour	Rump wetness	PCR result
13201	Zulu	22-Aug-16	clear	clear	clear	dry	Negative
13202	Yabba	22-Aug-16	clear	clear	clear	dry	Negative
13203	Willy-Wanda	23-Aug-16	clear	clear	clear	dry	Negative
13204	Vixen-Sierra	23-Aug-16	clear	clear	clear	dry	Negative
13205	Umunga	23-Aug-16	clear	clear	clear	dry	Negative
13206	Tana	23-Aug-16	clear	clear	clear	dry	Negative
13207	Silver	24-Aug-16	clear	clear	clear	dry	Negative
13208	Rio	24-Aug-16	clear	clear	clear	dry	Negative
13209	Quinnie	25-Aug-16	clear	clear	clear	dry	Negative
13210	Possum	26-Aug-16	clear	clear	clear	dry	Negative
13211	Olivia	27-Aug-16	clear	clear	clear	dry	Negative
13212	Neutron	27-Aug-16	clear	clear	clear	dry	Negative
13213	Muscles	29-Aug-16	clear	clear	clear	dry	Negative
13214	Lola	31-Aug-16	clear	clear	minor stain	dry	Positive
13215	Retreat	31-Aug-16	clear	clear	clear	dry	Negative
13219	Valerie	07-Nov-16	clear	clear	clear	dry	Negative
13223	Hillsie	21-Aug-17	clear	clear	clear	dry	Negative
13224	CC2	22-Aug-17	clear	clear	clear	dry	Negative
13225	Woody	22-Aug-17	clear	clear	clear	dry	Negative
13226	Clarkie	22-Aug-17	clear	clear	clear	dry	Negative
13227	Roadie	22-Aug-17	clear	clear	clear	dry	Negative

13228	Barmount	22-Aug-17	clear	clear	clear	dry	Negative
13229	Lotus	22-Aug-17	clear	clear	clear	dry	Negative
13230	Collaroy	22-Aug-17	clear	clear	clear	dry	Negative
13231	CR1	23-Aug-17	clear	clear	clear	dry	Negative
13232	CR2	23-Aug-17	clear	clear	clear	dry	Negative
13233	Connor	23-Aug-17	clear	clear	clear	dry	Negative
13234	Hail	24-Aug-17	clear	clear	clear	dry	Negative
13235	Crediton	24-Aug-17	clear	clear	clear	dry	Negative
13237	Longy	21-Aug-18	clear	clear	clear	dry	Negative
13238	Janine	21-Aug-18	clear	clear	clear	dry	Negative
13239	Professor	21-Aug-18	clear	clear	clear	dry	Negative
13240	Outerthere	22-Aug-18	clear	clear	clear	dry	Negative
13241	CCR41	22-Aug-18	clear	clear	clear	dry	Negative
13242	Moonlight	22-Aug-18	clear	clear	clear	dry	Negative
13243	Moonie	22-Aug-18	clear	clear	clear	dry	Negative
13244	Britto	23-Aug-18	clear	clear	clear	dry	Negative
13245	Eungy	23-Aug-18	clear	clear	clear	dry	Negative
13246	Barker	23-Aug-18	clear	clear	clear	dry	Negative
13247	Bob	23-Aug-18	clear	clear	clear	dry	Negative
13248	Cockie	24-Aug-18	clear	clear	clear	dry	Negative

### 4.3 Discussion

We recently conducted a systematic review of available information relating to disease presence and impact of chlamydial disease on koala populations throughout Australia (Grogan *et al.* 2017). Unusually, for such a high-profile species, there is a paucity of reports of systematic approaches to detecting disease in free-ranging koala populations and indeed there is a lack of population-level disease studies within the last two decades that examine mechanisms of chlamydial infection dynamics. There is now a pressing need for *in situ* comprehensive longitudinal population-level studies from diverse geographic regions, and the Clarke Connors Range koala population presents itself as an ideal target for one such study. Hence, we have chosen to utilize cutting edge diagnostic methods capable of distinguishing chlamydial species and strains to make sure we can provide information on role of chlamydial infection in this population, whilst also recording habitat, climatic and demographic data. Furthermore, wherever possible these samples have been retained for future use.

This section of the project is significant to our understanding of the role of chlamydial infection in free-ranging koalas in Queensland, because it is the first to target the prevalence and perhaps the impact of *Chlamydia* as a study focus in a relatively undisturbed population in Queensland. Although the landscape of the Clarke Connors Range includes highly modified and impacted areas, including roadsides and agricultural zones, our study attempted to sample koalas across the area, record disease signs and detect chlamydial infection. As a result, we are not limited to a smaller area (such as for the radio-tracking study) nor are we dependent on displaced koalas for our sample material (as are studies reliant on wildlife hospital admissions). Significantly, we surveyed across several state forests and often in areas of intact vegetation protected by farmers to decrease erosion or provide shelter for cattle, sampling koala populations away from urban development.

Although we consider our results to be preliminary, they are in stark contrast to the conclusions of studies in highly modified landscapes in south east Queensland (Craig *et al.* 2014; Waugh *et al.* 2016), where *Chlamydia* is considered to be a significant cause of population decline, driving the attempts to develop vaccines as a

putative management tool. We note that we will need to put our data in the context of that gathered from the Fauna Rescue Whitsundays Carers. The comparison of results eventually will be useful to understand the dynamics of disease in the region and may reveal more about the role of sampling methods (e.g. systematic population sampling v opportunistic sampling of sick koalas) in generating an understanding of this disease in koalas.

Our results strongly suggest that chlamydial disease is an insignificant factor in population dynamics in the Clarke Connors Range, a conclusion that aligns with results generated in the Brisbane Valley (Ellis 2015), Springsure district (Ellis *et al.* 1993), Clermont (Ellis *et al.* 2001) and St Bees Island (Melzer *et al.* 2013). Therefore, this result may not be so surprising, as chlamydial infection may require higher host population density for transmission and some other factors for expression, before it does impact population dynamics. Chlamydial infections are routinely asymptomatic with respect to the overt signs (ocular infection and “dirty tail”), but can still lead to infertility, so it was important to augment our observations and photographic record with PCR analysis of swab samples. These have (preliminarily) confirmed that the rate of chlamydial disease across the Clarke Connors Range is very low.

It is important to have context when discussing population health, and in this case, there are several other sites with which to compare the Clarke Connors Range. Compared to The Brisbane Valley, where despite relatively high fecundity, infection and disease were not uncommon, the Clarke Connors Range was typified by overtly healthy and *Chlamydia*-negative koalas. Over many years investigating the koalas inhabiting the Blair Athol and Clermont districts, our team found a contrasting picture emerge to that we appear to have uncovered in the Clarke Connors Range – very low occurrence of disease signs but a consistent underlying subclinical infection present in the population. Later in this report we also consider the Oakey population – at the lower end of the Brigalow Belt Bioregion: koalas at that site were either infected and appeared infertile or healthy and free of infection.

Further investigation into the population health of the Clarke Connors Range koalas is required, because we do not know whether we have simply found a widespread naïve population (yet to interact with the pathogen) that has been founded on koalas



that have not encountered chlamydial infection, whether this group has an innate capacity to deal with infection events (for example through immune responses) or whether our observation of low infection is the result of another, unknown situation. The presence in this region of diseased koalas presented to carers indicates that disease is clearly present, so a geographical examination of the extent of the disease, based on the wildlife carer data, is also warranted to assist. Finally, we have collected and provided to a leading laboratory blood samples from our collection, to assist the investigation of Koala Retrovirus (KoRV), which may have a role in the epidemiology of chlamydial disease in this population. Those samples are currently being analysed.

#### 5.0 Koala distribution and koala genetic relationships across the Clarke Connors Range

Anecdotal reports suggest that koalas are distributed broadly from the southern extent of the Clarke Connors Range through to the Eungella region in the north (Melzer *pers. comm.*). However, no systematic surveys of koalas specific to the Clarke Connors Range are recorded in the literature, so little is known about their actual distribution, health or abundance.

The landscape in this region includes a range of land uses and vegetation types dominated by agricultural activities but including patches of forest and woodland, eucalypt regrowth and vegetated riparian systems. Koala surveys and monitoring have been conducted to the west of this region (Clermont), and surveys by CQ University have also detected the distribution of koalas in the St Lawrence region, but little is known about the koalas inhabiting the Clarke Connors Range.

Our extensive surveying and koala radio tracking efforts in the Nebo region are complemented by road kill sampling on the Peak Downs Highway, but the tracking focused on a relatively narrow section of the Clarke Connors Range. As a result, we undertook a broader survey effort to sample the complete range, with the aim of determining the distribution of koalas and evaluating the prevalence of chlamydial infection and its impact across this region for koalas. This also allowed us to collect tissue samples for the genetic analysis required to examine relationships between koalas across the survey area.

Genetic diversity refers to the variability of genes in a species and determines the potential evolutionary fitness of a population and, ultimately, its long-term

persistence. In population genetics, the concept of heterozygosity is commonly extended to refer to the population as a whole, i.e., the fraction of individuals in a population that are heterozygous for a particular locus. It can also refer to the fraction of loci within an individual that are heterozygous. High heterozygosity (close to 1.0) means greater genetic variability, low heterozygosity (close to 0.0) means little genetic variability.

Gene diversity is composed of two elements:

- 1) the number of alleles at any loci; and
- 2) the abundance (or evenness) of the alleles.

If a population consists of an excess of homozygotes for different alleles this leads to a low observed heterozygosity but does not affect the expected heterozygosity calculated from Hardy-Weinberg Equilibrium. Generating the data to examine these concepts allowed us to run further tests to examine population structuring, based on genetic isolation or “distance” between individuals assuming that more closely related individuals would share more genetic material. The overarching purpose of this work was to detect any points of isolation or geographical areas of unique genetic material, so that future management of koalas in the Clarke Connors Range would be sensitive to the retention of all the genetic information (and hence evolutionary potential) of this population.

#### 5.1 Survey method and sample analysis

In order to cover as broad an area as possible with available resources, survey effort was primarily determined by accessibility; a road-based survey path was plotted using Google Earth Pro, and followed using an off-road vehicle and caravan as our research base. Potential locations were identified by referencing koala sighting data provided by Ian Gottke from Fauna Rescue Whitsundays Carers and Queensland Globe Vegetation Layer data for vegetation classification (Queensland Government). This allowed us to identify areas where koalas had previously been reported, and patches of vegetation containing probable koala habitat.

At each location, the three-person survey team undertook foot-based searches for signs of koala presence. An effort was then made to capture koalas at that location

before moving to the next potential site. Each evening, spotlighting surveys were undertaken on foot and in-vehicle to locate additional koalas.

Koalas were caught using the standard 'flagging' technique whereby they were encouraged to descend the occupied tree by waving a plastic bag above their head using telescopic metal poles - as described previously in this report. Once on the ground, koalas were restrained in a cloth bag and then anaesthetised while they underwent processing. This involved a basic health assessment, ear tagging, recording of body measurements, and collection of a DNA biopsy and swab samples. Swabs were later analysed in the laboratory using a quantitative PCR approach to diagnose chlamydial infection as described earlier in this report. The capture and sampling process took between 15-30 minutes, after which koalas were allowed time to recover from anaesthesia and then returned to the same tree from which they were captured.

Genetic analysis of the tissue samples was completed using a 20+ microsatellite panel developed at the University of Queensland (UQ), and based on previous markers identified by Houlden *et al.* (1999), and Cristescu *et al.* (2009), as well as unique markers identified by the UQ research team (Hulse, Ellis *et al* unpublished data). The microsatellite panel allowed us to interpret our results in the context of several other Queensland koala populations we are also investigating. In the near future these data will inform discussions of the local, regional and state wide and national patterns of genetic connectivity amongst koala populations (Dudaniec *et al.* 2013). All DNA extraction and amplifications were undertaken in the laboratory at UQ.

Analysis of genetic diversity was performed using the software GENALEX, version 6.5 (Peakall & Smouse 2006) to calculate mean number of alleles and observed and expected heterozygosity. FSTAT (Goudet 2001) was used to calculate allelic richness using standardisation, to allow comparison between groups and inbreeding coefficient which provides a relative measure of relatedness compared to a random system. Inbreeding coefficients can also be used to indicate the proportion of shared ancestry in pairs of individuals. Expected values are  $\leq 0$  for unrelated individuals, 0.25 for half-sib pairs and 0.5 for parent-offspring or full-sib pairs (sharing 50% DNA). Relatedness values form a distribution around these expected values.

The value “ $F_{ST}$ ” reports population genetic differentiation by calculating the proportion of variance in allele frequencies among each population relative to the total variance. As a measure of genetic differentiation within populations,  $F_{ST}$  is calculated to estimate the genetic distance between survey sites, and our original strategy was to utilise this across the Clarke Connors Range to assess variability between the groups. The greater the genetic distance between groups or populations, the less gene flow we would assume to occur between them and the more isolated they are considered to be from one another.  $F_{ST}$  can range from zero to one, where zero means complete sharing of genetic material and one means no sharing. However, as we have discussed, there were no significant breaks in koala distribution in our surveys, so rather than clumped groups of samples we have more of a continuum across the study area, which does not facilitate  $F_{ST}$  at this site, although we were able to assess it between the Clarke Connors Range and the other sites we simultaneously surveyed across Queensland. The vast, dispersed nature of the Clarke Connors Range population served also to confound population analyses, since a population is regularly considered to be a group of individuals existing within sufficiently close proximity that any two individuals may reproduce (i.e. random mating). A population may exist as a single large population (where mating is random) or multiple subpopulations with varying levels of connectivity, and the outcomes of both from a genetic standpoint can be quite dissimilar. Genetic diversity is best conserved in a single large population and is more at risk of being lost where a population is fragmented, existing as multiple smaller subpopulations. Nonetheless, population structuring was determined using the Bayesian clustering program STRUCTURE version 2.3.4 (Pritchard 2000). Analysis of koala population genotype data involved 5 replicates of  $K = 1$  to  $K = 10$  ( $K =$  genetic cluster) using 100,000 iterations with 100,000 iterations discarded as burn-in. The number of  $K$  clusters was determined using both the maximum likelihood and the deltaK method of Evanno *et al.* 2005.

## 5.2 Results

### 5.2.1 Summary of sites surveyed and koala captures

In addition to the intensive surveys and monitoring undertaken in the Nebo district, designed to build the group of koalas for intensive monitoring using GPS/VHF collars, three larger-scale surveys following the Clarke Connors Range were

undertaken, in August 2016, 2017 and 2018. In 2016, a south-bound survey of several days identified locations at which surveys were deemed likely to result in koala capture, and in 2017 and 2018 trips of 5 to 10 days were undertaken between Marlborough in the south and Eungella Dam in the north.

Koala surveys were undertaken across a variety of areas including road verges, sparsely wooded grazing country and densely vegetated creek systems throughout the Range system. Koalas were found at each location (see figure below), being particularly abundant in the Nebo district. Koalas were found on the several properties to the east and west of Nebo where koalas were also commonly spotted by local landowners. At some sites, we were unable to survey for koalas, generally because we were unable to co-ordinate a suitable time with the landowners, but in general a wide survey area was covered with an adequate sample of koalas to undertake our genetic and disease studies.



Figure 37. Koala capture sites across the Clarke Connors Range 2016 – 2018. Numbers refer to individual koalas as identified in this report and used in the genetic analyses.

Koalas were sighted during travel along formed roads, during the on-ground day time surveys and during night surveys using spotlighting. Our ability to remain mobile and establish a camp at any location during surveys meant we could systematically survey sites without the requirement to remain close to towns for supplies. All samples were kept frozen in our field laboratory.

In both broad-scale surveys (2017 and 2018), we first encountered koalas at the road crossing of Clarke Creek (-22.638879, 149.259144), which included a vegetated riparian zone and some large eucalypts within the road reserve. Koala 13223, an adult female (“Hillsie”) with large back rider was observed and captured at this location in 2017. During processing this koala, contact was made with the adjacent landowner who granted the team permission to enter his property and conduct further searches of Clarke Creek.



Figure 38. Field processing, Clarke Creek, Qld.

Further searches of the area uncovered several more koalas, including four females (all with back young) and one male, which were detected during spotlighting surveys. Spotlight surveys in 2018 revealed a number of females with young, many greater gliders (*Petauroides volans*) and several tagged koalas that had been captured the previous year.

Over the course of our surveys, the team travelled north to Nebo from Marlborough: in 2017 through Lotus Creek, Collaroy and the Connors River and in 2018 heading through Blue Mountain, surveying for and catching koalas at the locations identified in Figure 37.

From Nebo, surveys were conducted north to Hail Creek Mine, Collinsville and then across Lizzie Creek Road through Crediton State Forest to Eungella Dam. In 2017 we surveyed into Eungella township, searching to Bee Creek from Broken River (the later surveys being unsuccessful, but koalas were captured near Hail Creek and in Crediton State Forest). In 2018, state forests near Mt Britton, the Moonlight Dam access and Mt Barker to the north-west of Eungella Dam were investigated, with several additional koalas located and sampled. All koala captures are identified in Figure 37 and details of captures during this survey appear in Table 3.

The koala population in the Clarke Connors Range appears broadly distributed and in good health. We recorded fecundity at some 73% across the two years, but with a) gender unattributed in almost half of the observations of independent adults and b) small pouch young unlikely to be detected without capturing the mother, the confidence in this estimate is low. Spotlighting for koalas will often result in koalas being found high up amongst foliage, and their gender cannot always be ascertained. Unless pouch, testes or sternal gland could be clearly seen, observations were assigned to “unconfirmed”. Significantly, some pouch young were quite large, so it is possible that some undetected, semi-independent young were also present, which would also likely skew this result.

Our surveys were designed to gain a snapshot into the koala population, and a further systematic survey of this region is recommended to fully understand the distribution and abundance of koalas in the Clarke Connors Range. During our second survey, we concentrated more effort to the area north of the Peak Downs Highway, and were rewarded with koala sightings where previously none had been recorded. Even so, several roads were still impassable for caravans so our surveys were not as extensive or comprehensive as possible in better weather conditions.



Figure 39. Field laboratory in situ, Crediton State Forest.

Significantly, we were able to locate koalas north west of Eungella Dam and in the Crediton State Forest, fulfilling our aim of detecting and sampling koalas from these areas. Whilst camping it was not uncommon to detect koala bellows in this landscape, a positive sign of population health.

#### *5.2.2 Distribution and genetics*

During the 2017 survey, 42 koalas were observed, comprised of 33 independent koalas and nine young. Of the 33 independent koalas, seven were confirmed as male and 11 as female, while gender could not be determined for the other 15. We successfully captured and examined 13 koalas, not including young. Of the 11 adult koalas that were confirmed as females, nine (82%) had back or pouch young that were observed.

In 2018, we detected 37 independent koalas, of which ten were captured, comprised of six males and four females of which two had back young. Overall, gender was confirmed for 20 adult individuals, of which nine were male and 11 were female. Of those 11 females, seven (64%) were observed to be carrying young.



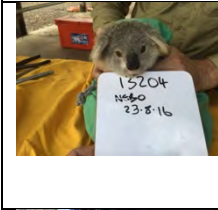



Photographs of the head and rump of captured koalas provide key source material for comparison with disease testing results. All koalas are uniquely tagged (ear tag) for future reference and in case they are subsequently encountered. Each koala also has a unique database number (University of Queensland number) associated with any samples collected at the time of capture. Table 4 provides a basic overview of













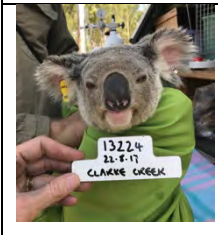




each koala we caught; detailed information is contained in further tables and data summaries (for example for koalas that were monitored using VHF tracking, or for whom DNA and Chlamydial PCR data are provided in this report).







As the images indicate, we found the koalas of the Clarke Connors Range to be generally in good health. Instances of chlamydial disease appear more likely to be detected in the Nebo district (see population health section of this report).










Table 4. Summary of adult or semi-independent koalas captured and sampled for this study.

	<p>Koala 13201 (“Zulu”) first captured 22-Aug-16. Female Adult 7 kg with moderate tooth wear (line) and no sign of disease. Caught with young – 13202 (Yabba - not pictured).</p>
	<p>Willy Wanda, 13203 first captured in August 2016 as a 5.5 kg female with body score 7.5. Her young was 13204, a female.</p>
	<p>Vixen-Sierra was captured with mother Willy Wanda also in August 2016 and was resighted throughout the study, eventually sitting away from the mother. One kg at first capture, she was always in the same tree as 13203.</p>
	<p>13205 “Umunga” first caught 23-Aug-16. Female Adult 5.7 kg with minor tooth wear and body score of 8. Back young (13206 - male).</p>
	<p>13206 – male back young of 13205, body mass 0.95 kg at capture (23-Aug-16). Body score 9.</p>
	<p>13207 “Silver” caught 24-Aug-16. Female Adult 5.51 kg, slight tooth wear and body condition 7.5. No sign of disease.</p>

	<p>13208 was a 9 kg male captured as part of an additional study, but in the vicinity of Nebo, hence his morphological data, genetic and disease information has been included here. Similarly, many samples coming from wildlife carers will be included in the larger study of genetics, but are not reported here.</p>
	<p>13209 "Quinnie" caught 25-Aug-16. Female Adult 5.00 kg, tip wear on premolar, body condition 7.5.</p>
	<p>13210 "Possum", caught 26-Aug-16. Female adult 5.0 kg, no tooth wear, body score 8, eyes and rump clear.</p>
	<p>13211 "Olivia" first caught 27-Aug-16 near Nebo. Female adult body mass 4.59 kg, no tooth wear body score 8.</p>
	<p>13212 Male adult "Neutron", first caught 27-Aug-16 Body mass 5.54 kg, slight wear tips premolar, body score of 9.</p>
	<p>13213 Muscles, another large male that presented with an injured paw, not deemed likely to have a negative long term impact, but sufficient to warrant us excluding him from the tracking study. 7.8 kg at capture, with major sternal gland activity.</p>
	<p>13214 female adult "Lola" first captured 31-Aug-16 Body mass 6.79 kg, premolar full ring of wear, with some wear on M1. Young is 13215.</p>

	<p>13215, male young of Lola, named “Retreat”, captured 31-Aug-16 at 2.37 kg, no tooth wear and body score 8.</p>
	<p>Valerie was first captured in April 2017 and was observed regularly using trees of the <i>Lophostemon</i> genus. Her range tracked the Peak Downs highway, rarely crossing. At 4.5 kg, body score of 7 and some tooth wear (2 lines) she had a small “pinky” in her pouch at first capture, which she reared successfully while being monitored.</p>
	<p>13223 “Hillsie”, female adult captured at Clarke Creek 21-Aug-18, no signs of disease, found with back rider (not captured). 6.5 kg body score 6.</p>
	<p>13224 “CC2” captured at Clarke Creek on 22-Aug-17 Female, adult 5.66 kg, tip wear on PM, Body score 7, mother of 13225.</p>
	<p>13225, male young of 13224. “Woody”, caught near Clarke Creek on 22-Aug-17. 1.76 kg, no tooth wear, body score 10</p>
	<p>13226 “Clarkie”, caught 22-Aug-17. Male adult 9.34 kg, 2 lines of wear on premolar, body score 7.5, sternal gland active.</p>
	<p>13227 “Roadie”, captured adjacent to Marlborough Sarina Rd on 22-Aug-17. Male adult 7.20 kg, tip wear on premolar, body score 8.</p>
	<p>13228 “Barmount”, caught 22-Aug-17. Female adult 6.61 kg, tip wear only on premolar, body score 7.5. Young present (not caught).</p>

	<p>13229 “Lotus”, caught 22-Aug-17. Separated from mother (not captured) in same tree. Male, body mass 1.32 kg, body score 8.</p>
	<p>13230 “Collaroy” captured on forest access Rd on 22-Aug-17. Female adult 4.74 kg, nil tooth wear, body score 7.</p>
	<p>13231 “CR1” Captured 23-Aug-17. Female adult, 7.12 kg, full ring of wear on premolar, body score 6.5. Caught with young (13232).</p>
	<p>13232 “CR2”, 1.98 kg female back young of CR1, captured on 23-Aug-17. Body score of 8.5.</p>
	<p>13233 “Connor” captured 23-Aug-17. Male adult 6.94 kg, tip wear on premolar, body score of 6.</p>
	<p>13234 “Hail”, Adult male captured near Hail Creek Mine on 24-Aug-17. Body mass 5.84 kg, tip wear on premolar, body score of 7.5.</p>
	<p>13235 “Creditor” captured near Lizzie Creek Road Creditor State Forest on 24-Aug-17. Male adult 9.05 kg, premolar tip wear only, body score 7.</p>
	<p>13237 “Longy” captured from road verge on 21-Aug-18. Female adult at 3.98 kg, tip wear on premolar, body score 7.5.</p>
	<p>13238 “Janine”, Adult female with young (13239) caught at rodeo ground, Clarke Creek on 21-Aug-18 Body mass 5.28 kg, premolar ring of wear, body score 5.5</p>
	<p>13239 “Professor”, male back young of 13228 caught 21-Aug-18. Body mass 0.96 kg, no tooth wear and body score of 9.</p>

	<p>13240 "Outerthere", male adult of 7.8 kg, caught 22-Aug-18. Two lines of wear on premolar, body score 7, caught 1 km north of Connors River</p>
	<p>13241 "CCR41", caught 22-Aug-18. Male sub adult (4.07 kg) with minor tip wear on premolar and body score of 7.</p>
	<p>13242 "Moonlight" caught near Moonlight Dam on 22-Aug-18. Male adult body mass 9.06 kg, premolar two lines of wear, body score 6. Night catch.</p>
	<p>13243 "Moonie", caught at night on 22-Aug-18. Male adult, 5.61 kg, tips worn on premolar, body score 8.</p>
	<p>13244 "Britto", caught 23-Aug-18. Female adult 6.26 kg full ring of wear on premolar, body score 7.</p>
	<p>13245 "Eungy" caught near Eungella Dam on 23-Aug-18. Female adult body mass 5.61 kg, with young 13245. Body score 6.5.</p>
	<p>13246 "Barker", caught with mother (13245) on 23-Aug-18. Male back rider 1.32 kg, no tooth wear, body score 9.</p>
	<p>13247, "Bob" was caught near Eungella dam in August 2018. This adult male in good condition (body score 6, 7.53 kg) had no signs of disease but some tooth wear indicating an age over 5 years.</p>
	<p>13248 "Cockie" was caught near Cockies Creek Rd in August 2018, a male adult (7.55 kg) with no signs of disease and a body score of 8.</p>

Genetic analysis for this project included 149 koala samples, of which 54 samples were collected from the Clarke Connors Range. These were compared against 39 from Mt Byron in south-east Queensland, 16 from the Oakey district and 40 from St Bees Island. Microsatellite genotypes across 29 loci were generated for all animal samples.

Genetic diversity values, estimated through expected heterozygosity and allelic richness compared between all koala populations (Table 5) reveals similarity between all populations, with the exception of St Bees which has the lowest diversity for both allelic richness and expected heterozygosity.

Table 5. Summary of genetic diversity statistics for koala populations compared with the Clarke Connors Range

<b>Region</b>	<b>N</b>	<b>A<sub>mean</sub></b>	<b>A<sub>r</sub></b>	<b>F<sub>IS</sub></b>	<b>H<sub>o</sub></b>	<b>H<sub>e</sub></b>
Clarke Connors	54	9.38	5.32	0.210	0.592	0.740
Mt Byron	39	8.07	4.86	0.135	0.621	0.706
Oakey	16	6.28	4.58	0.081	0.637	0.668
St Bees	40	6.72	3.92	0.151	0.533	0.619
Total (or mean of samples)	149	7.359	5.48	0.152	0.603	0.696

**N**: Number of individuals sampled. **A<sub>mean</sub>**: Mean number of alleles. **A<sub>r</sub>**: Allelic richness. **F<sub>IS</sub>**: analysis of inbreeding coefficients across the populations we have included here, **H<sub>o</sub>**: Observed heterozygosity. **H<sub>e</sub>**: Expected heterozygosity. Inbreeding coefficient being the proportion of variance in a population that is contained within an individual ( $F_{IS} > 0.00$  suggests inbreeding).

Our analysis of genetic variability within and between populations ( $F_{ST}$ ) appears in Table 6, where values range from 0 to 1. A zero value should mean the two comparison populations are interbreeding freely. Conversely, as we approach a value of 1, the two populations share less genetic diversity. From these results, there appears to be considerable sharing of genetic information across the groups of koalas we have included in this study.

Of most importance to this study was the structuring of the koala population within the Clarke Connors range, wherein we found two groups, which we have simply classed as haptype 0 and haptype 1.

The most common haptype (0) was spread across the Clarke Connors Range, with the majority of koalas from the majority of survey locations conforming to this specific genetic suite.

Table 6. Pairwise  $F_{ST}$  values among koala survey locations.

	Mt Byron	Oakey	St Bees
Clarke Connors	0.093	0.104	0.082
Mt Byron		0.079	0.164
Oakey			0.176

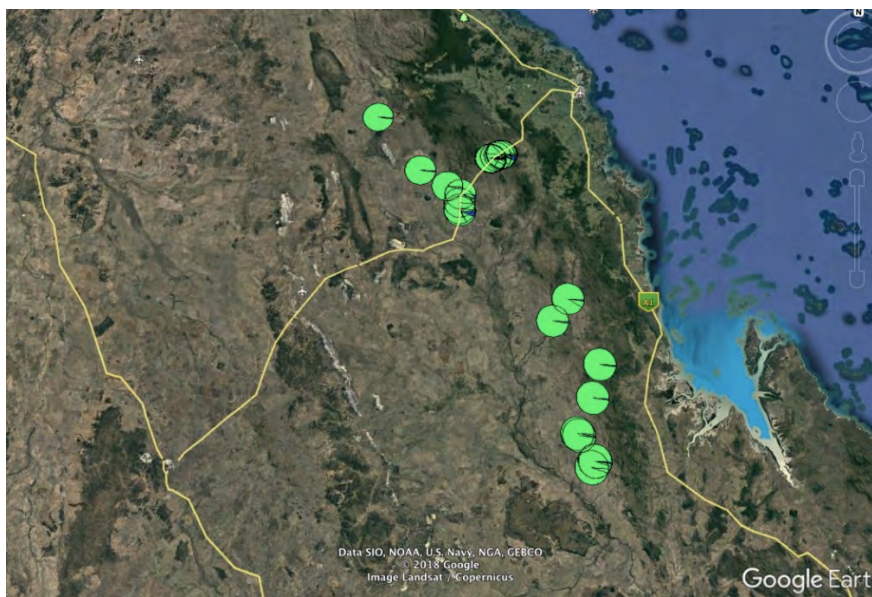


Figure 40. Haptype 0 distribution, Clarke Connors Range



Figure 41. Haptype 1 distribution, Clarke Connors Range

A further distinct genetic group was predominantly restricted to the broader Nebo district (Nebo to Mt Spencer at least), with limited sharing of alleles further afield. These preliminary data from a relatively limited sample are held at the University of Queensland (W. Ellis pers. com.) where research is ongoing.

### 5.3 Discussion

Our genetic analysis is preliminary, having collected the last of the tissue samples in August of 2018 and only recently run the extraction and begun analysis of this tissue. As a result, we can present all the genetic data for the Clarke Connors Range (Appendix 2), but we are still interpreting its ramifications for the genetic makeup of this study region and the broader implications for this population.

The Clarke Connors Range koala population appears genetically diverse, having comparatively (if not statistically) more allelic diversity than the other groups we looked at; however, our sample is potentially biased by the greater number of samples collected across this study region (and its size) by comparison to other sites. This is also true for the geographical extent of each group, with St Bees Island, founded on some 17 koalas almost 100 years ago, still presenting high genetic diversity despite the long period of isolation and small founding group.

A key goal of our study was, however, to investigate the geographical spread of genetic information across the Clarke Connors Range, to generate information that could inform management of this group. This element of our work is more complete



and although our analyses have identified two groups of koalas, based on genetic dissimilarity, the spatial delineation of these groupings does not suggest the presence of a significant and long-term barrier to gene flow across the Clarke Connors Range, nor the natural separation of the population into more than one geographic unit, on the basis of genetics.

The genetic relatedness amongst koalas within the Clarke Connors Range is presented in Appendix 2, and figures 9 and 9a, which allows cross checking against known mother offspring pairs (Appendix 2) and allows some understanding of the relatedness of geographically close individuals across the survey area.

We have generated data that will allow us to investigate the genetics of the koalas of the Clarke Connors Range at a landscape - genetics scale. By doing this, we hope to provide data that will feed into larger koala genetics studies across Queensland and Australia to:

- a) help tease out the geographic scale at which management decisions need to be made; and
- b) provide some insight into the population genetics of this group to detect any local processes acting on the group across its range.

We have also begun the process of collecting and analysing samples from across Queensland to determine where the genetic boundaries (if any) of this population lie. The significant movement of koalas we discovered during our tracking work suggests that there will be a large amount of gene flow across the borders of the Clarke Connors Range. We hope to eventually detect areas of significantly reduced gene traffic between the predominant Clarke Connors Range genotype (or most common alleles that differentiate this group from others) and the closest geographically placed but genetically differentiated groups. This is a relatively controversial approach, as we are asking questions of the genetic information from an ecological and evolutionary standpoint, using simple math and logic. In essence, the movement of genetic information between localities of koalas should reflect the functional connectivity of the landscape for koalas, but this can be confounded by many historical influences that we, as a research team, may overlook.

We have previously relied on isolation by distance to investigate population level genetic differentiation (Ellis *et al.* 2002a), but recent work has focussed on isolation by resistance which appears to be an inherently superior approach, as long as one

can locate or define the groups to examine. This approach has been used for koalas in south east Queensland (Dudaniec *et al.* 2013) providing key tools for conservation in that region. For the Clarke Connors Range koalas, there may be no functional resistance – even cleared agricultural areas, large distances across ranges and linear infrastructure apparently proving to be ineffective barriers to koala movement.

A fundamental problem all studies that use genetic markers to detect population boundaries face, is that the scale of observations and the temporal and spatial inconsistency inherent in ecological surveys – such as ours – can lead to inaccurate conclusions about the sensitivity of a species to particular events or landscape features (Anderson *et al.* 2010). As a result, we have taken a very cautious approach to interpreting the data at this stage. There are two genetically distinguishable groups amongst this population, but their spatial overlap is significant. Koalas from any sampled region of the Clarke Connors Range may have considerable genetic similarity with any other region, but within the samples we did find separation, which was at its greatest in the broader Nebo district. A grouping of koalas from the Nebo district shared little genetic similarity with other areas, but these koalas co-exist with others with high similarity to what may be considered to be the representative Clarke Connors Range genotype. This conundrum could be resolved with the addition of further samples from adjacent populations, such as west to Clermont and south through the Biloela – Monto districts, and we also hold samples from the St Lawrence locality. We are currently developing an approach that will utilise these samples and historical genetic data to establish the significance of the population structuring we have found in the current project. It is equally likely that a unique genotype has colonised or mutated within the broader Nebo district at some time in history and that the structuring we have seen reflects the descendants of this event.

## 6.0 Permits

This project operates under CQ University Animal Ethics Committee Approval 20182 and Queensland Department of Environment and Heritage Protection Scientific Purposes Permit WISP17479316.

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## Appendix 1. PCR results for koalas of The Clarke Connors Range

Koala ID	Swab site	23S <i>Chlamydia</i>	<i>C. pecorum</i>	<i>C. pneumoniae</i>	<i>Mycoplasma</i>	<i>Ureaplasma</i>	<i>Bordetella</i>
13234	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13234	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13234	UGT	NR	NR	NR	NR	NR	NR
13235	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13235	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13235	UGT	NR	NR	NR	NR	NR	NR
13231	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13231	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13231	UGT	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13232	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13232	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13233	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13233	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13233	UGT	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13224	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13224	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13224	UGT	NR	NR	NR	NR	NR	NR
13225	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13225	R EYE	NR	NR	NR	NR	NR	NR
13226	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13226	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13226	UGT	NR	NR	NR	NR	NR	NR
13227	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE
13227	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13227	UGT	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13228	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13228	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13228	UGT	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13230	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13230	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13230	UGT	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13223	L EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13223	R EYE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
13223	UGT	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE

