

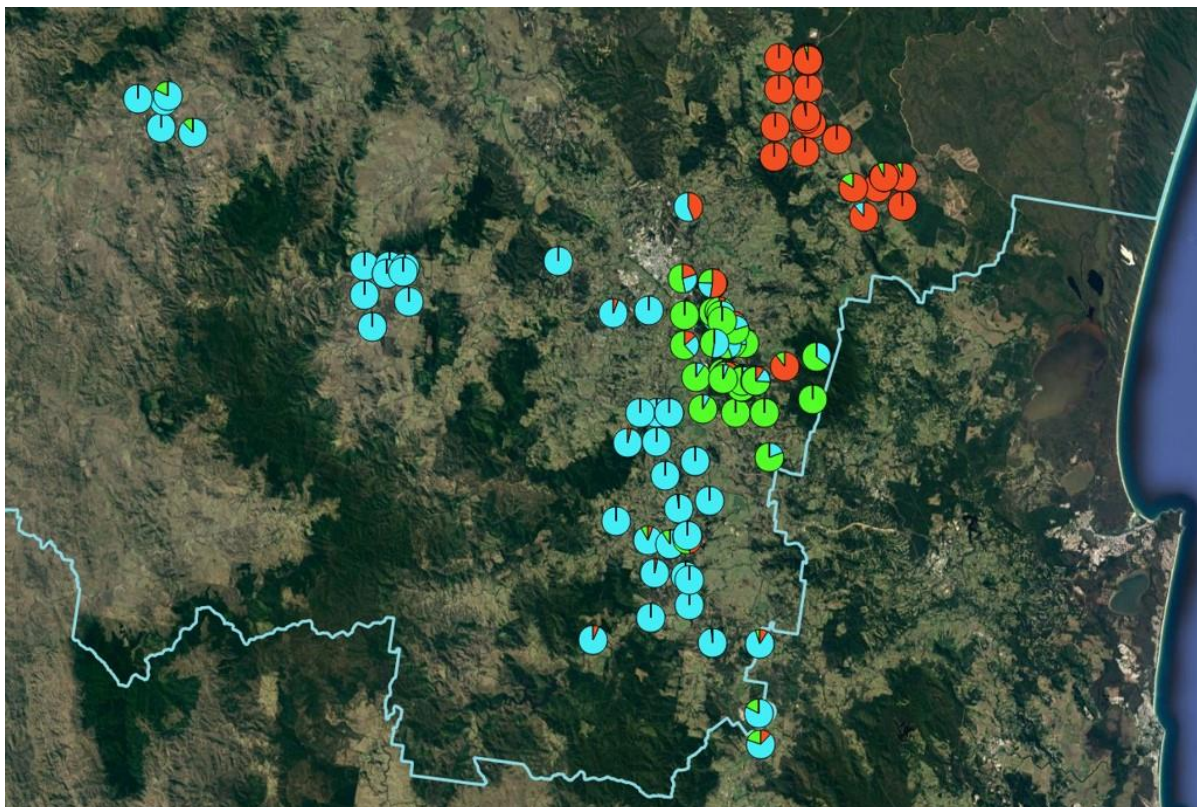
## Appendix 3. Genetic analyses of the Gympie koalas

### Methods

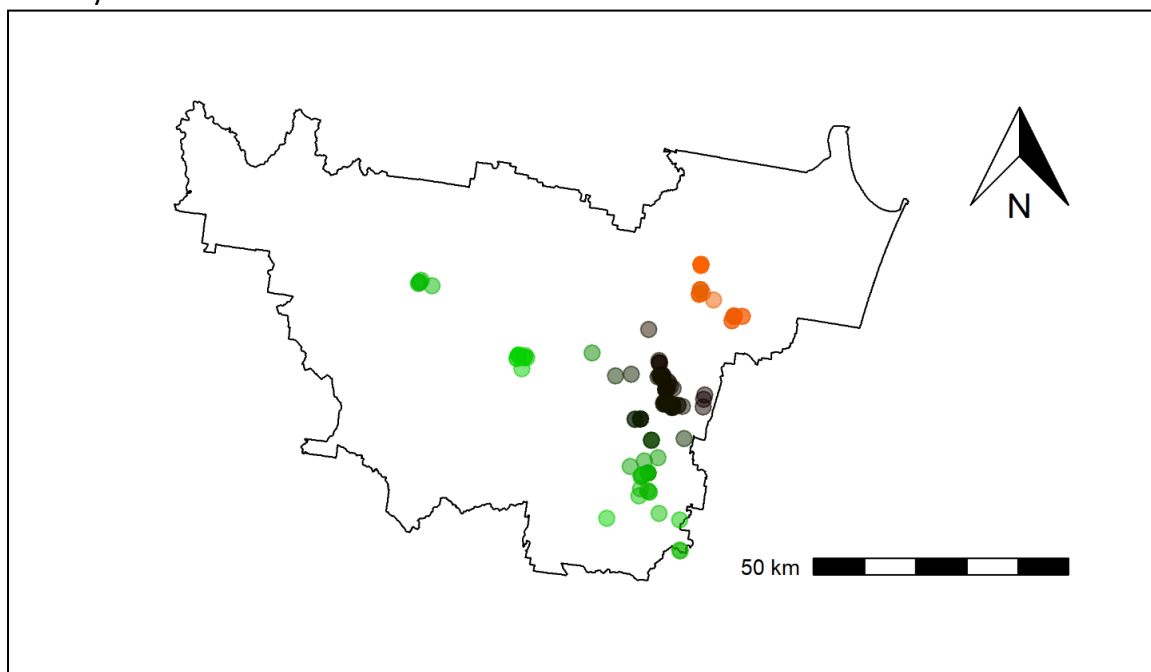
USC undertook koala scat surveys using detection dogs in the Gympie Regional Council for the purpose of genetic analyses. The DNA analyses of scats successfully identified 129 koala scats within a 33km radius surrounding the Section C Project. Fifty of those scats were identified within the 2 kms impact zone of the Section C Project (see Figure 1 of the report). To assess the general population structure of koalas in Gympie Regional Council area, two analyses were run. First, a variational Bayesian framework implemented in the software fastSTRUCTURE (Raj et al. 2014) was used to calculate an estimate of the most likely number of ancestry populations. Second, a spatially explicit multivariate method (sPCA) which identifies non-random spatial distribution of genetic variation (Jombart et al. 2008) was used to identify fine-scale population structure. The sPCA analysis was implemented in the R package adegenet (Jombart 2008), with the neighbourhood by distance approach, setting the minimum distance to 0 meters and the maximum distance to 10,000 meters, which reflects the expected maximum dispersal distance for koalas (Dique et al. 2003).

### Findings

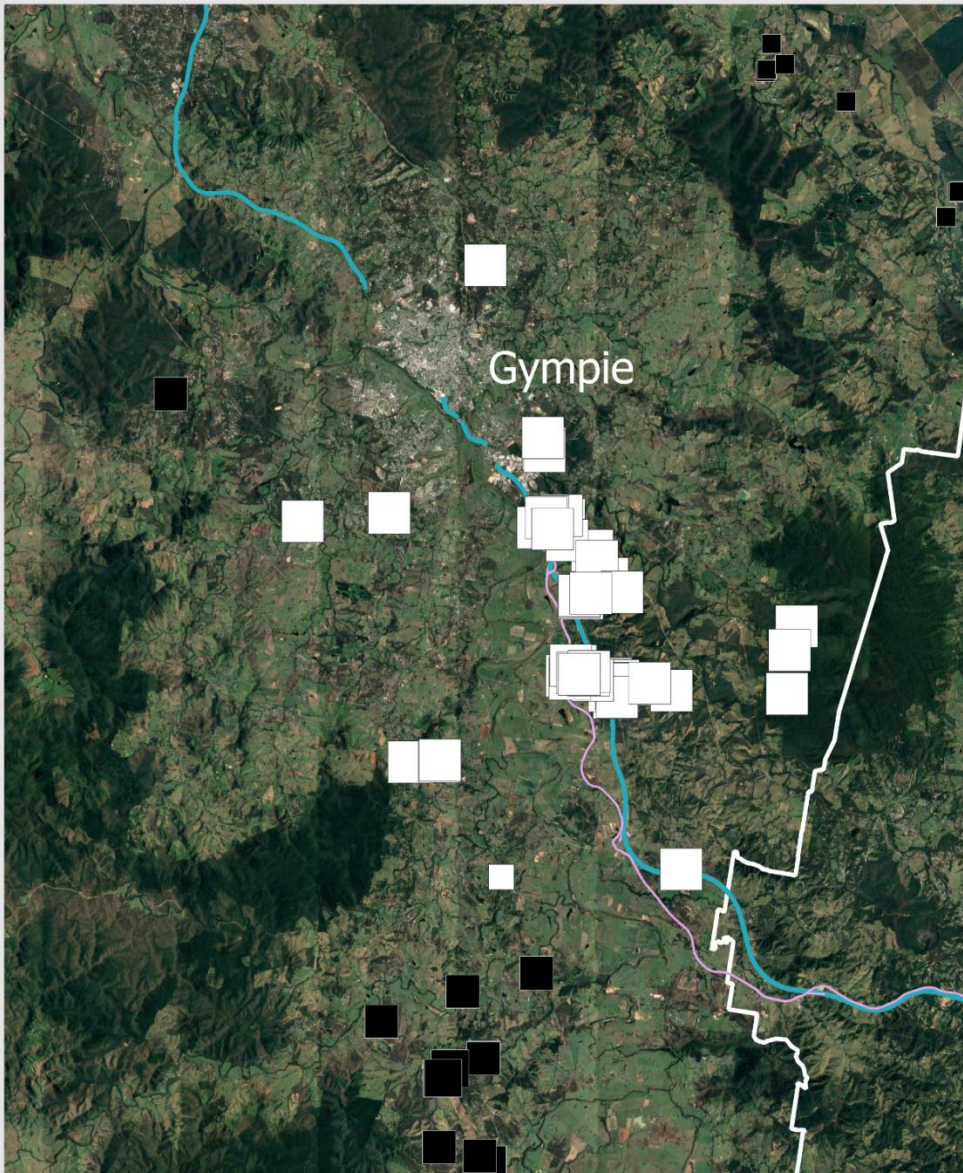
Both Faststructure and sPCA analyses indicated the presence of fine-scale population genetic structuring among koalas in the Gympie Regional Council area (Appendix 13, Figure 1 and 2). Koalas around the Kybong/Traveston (represented by the predominately green pies) area showed some level of genetic differentiation from koalas in Goomboorian (represented by the predominately red pies) and to the far west and south (represented by the predominately blue pies). There was, however, evidence of some extent of gene flow between these sub-populations, as seen by the multi-colouration (mixed red, green and blue) of some pies across the studied landscape. However, both set of analyses indicated that koalas within the 2 kms impacted zone showed some extent of genetic differentiation from the rest of the koalas in the Gympie Regional Council area. For now, our genetic analyses showed that koalas on both sides of the highway within the impacted zone still form one connected genetic pool (represented in white; Appendix 13 Figure 3). Results highlight the importance of maintaining the genetic connectivity between both sides of the highway to minimise increased risk in genetic erosion in the long-term.



**Appendix 3, Figure 1.** Fastructure identified the presence of three genetically distinct groupings across the Gympie Regional Council area. Pies are GPS referenced to the scat and colours indicate the scat genetics' identity.



**Appendix 3, Figure 2.** sPCA analysis indicates the presence of 3 genetically distinct populations across the Gympie Regional Council area as identified using Faststructure. The colours of the pies indicate the koala scats' genetic identity.



**Legend**

- Individual koala
- Gympie LGA boundary
- Bruce Highway
- Old Bruce Highway



Map Projection: Universal Transverse Mercator  
Horizontal Datum: WGS 84

While every care has been taken to ensure accuracy of the data, USC does not warrant that this information is definitive or free of errors and does not accept liability for any loss caused or arising from reliance upon information provided herein.

**Appendix 3, Figure 3.** This map shows a closeup of the Section C Project with the second principal component of the sPCA plotted. The different colours indicate differentiation, and the larger the squares, the more different these koalas are to each other. This figure indicates that koalas within the Impact zone are genetically one population.