WHAT DO KOALAS EAT WHERE AND DOES THIS SHAPE THEIR MICROBIOMES?

Background and significance of the topic

Throughout their wide geographic range, koalas always eat *Eucalyptus* leaves. Few animals can stomach let alone survive on these fibrous, toxic leaves and koalas are thought to rely on their gut microbiomes to help them digest their nutritionally poor diets. Our research has revealed that the microbes that make up the koalas' gut microbiomes vary over their range with koalas from proximate populations having more similar microbiomes compared to koalas from opposite ends of the continent. These patterns extend to the microbes' ability to breakdown and make different compounds, thereby, potentially influencing the koalas' nutrition. Our work suggests that climate and dispersal patterns play a role in shaping these patterns but we don't know how diet is involved.

Within one population we found that individuals that feed on different eucalypts have distinct microbiomes, while those with similar diets have similar microbiomes. Yet, the microbiomes of koalas on St. Bee's Island differ from those on North Stradbroke Island, despite koalas on both islands reportedly feeding on *Eucalyptus tereticornis*. By contrast, koalas at Clermont, Surat and Gunnedah have similar microbiomes and are thought to have similar diets. To resolve these discrepancies we need accurate information on which species the koalas are eating.

Precise questions to be addressed (objectives)

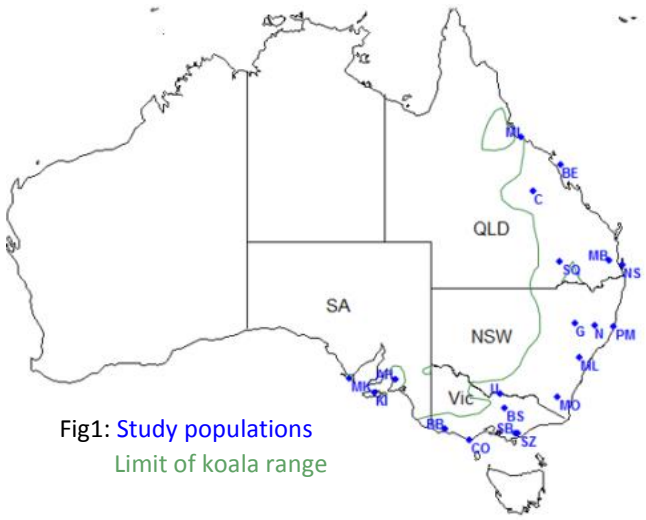

Fig1: Study populations
Limit of koala range

This study will leverage extensive pre-existing data, samples and significant prior funding to determine how the species and nutritional composition of the koalas' diets affects the composition and function of their microbiomes across populations. To do this we will identify which species of *Eucalyptus* koalas are eating at sites across Australia, including St. Bee's Island and Clermont in Central Queensland (Fig 1). A better understanding of koala diets will assist identification, conservation and restoration of their habitat. Further, understanding how koala diets interact with the microbiome to influence koala nutrition is vital to assist rehabilitation and translocation of koalas.

Approach, study design

We will characterise the diet (eucalypt species eaten) of 187 koalas from 20 populations across four states (Fig 1). This will allow us to compare koala diets across the country and to connect koala nutrition and microbiome composition. The nutritional composition of the koalas' diets will be determined from data on the chemical make-up of the eaten eucalypt species. This information will be combined with existing data on the composition and function of the animals' microbiomes (Fig 2).

As part of a completed ARC grant, we have already collected the required samples and characterised the koalas' microbiomes by shotgun sequencing of DNA extracted from faecal pellets. We have also characterised the nutritional composition of candidate eucalypt species from the areas where the koalas were sampled using near-infra-red spectrometry (NIRS) and chemical analysis. However, determining the composition of the koalas' diets was beyond the scope of that project and required the development of new genetic techniques.

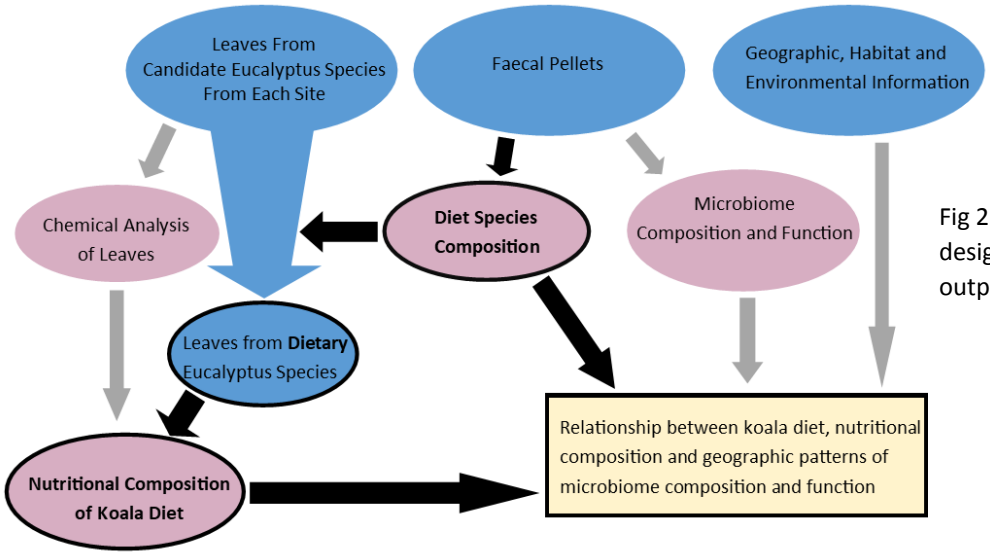

Fig 2: Flowchart of study design. Black outlines indicate output from this study.

Identifying what koalas eat in different parts of the country is trickier to answer then one might think. For one, koalas often feed at night in different trees to those that they rest in during the day, making direct observations difficult and labour intensive. Other researchers have used microhistological analysis of leaf cuticle fragments and chemical analysis of hydrocarbon cuticle waxes from faecal pellets to reconstruct diets, but neither method is able to differentiate between many eucalypt species. Standard genetic approaches to faecal diet analysis (e.g. DNA barcoding) do not work for eucalypts due to their genetic similarity. We have developed a new approach that utilises state-of-the-art next-generation sequencing to characterise koala diet composition.

Method to be used

We have previously sequenced 4 trees of each candidate *Eucalyptus* species from each site using DArTSeq (Diversity Arrays Technology Sequencing), a next-generation sequencing (NGS)-based method that uses restriction enzymes to produce reduced representations of genomes. In this study, we will use this sequencing data to identify small sequences of DNA ("tags") that are unique to each candidate species. Another DArT technology, DArTag, will be then be used to selectively amplify these "tags" from DNA extracted from our previously collected koala faecal pellets. By matching the sequences of the amplified "tags" to our candidate species reference set we will identify the species eaten by the koalas. We will also determine which species are major or minor components of the koalas' diets from the sequencing read counts. A trial of this technique at two study sites has yielded promising initial results.

Timelines for undertaking the project

We will design and optimise the species specific "tags" over the first 6 months of the project. The faecal samples will then be sequenced over the following 6 months. A peer reviewed article reporting the geographic patterns of koala diet composition will then be compiled for publication in a journal such as *Austral Ecology*. Further, the data will subsequently be combined and co-analysed with our existing microbiome and leaf nutritional composition data to produce a high impact publication in an international journal such as *ISME*. The funds of $5000 dollars from this grant will be used in the payment of sequencing costs at Diversity Arrays Technologies. Without these funds the diet composition analysis will not be possible.

Capability of the applicants to achieve the outcome

Dr. Michaela Blyton, a research fellow at the University of Queensland, will be the chief investigator on this project and will undertake the primary data analysis. Dr. Ben Moore, a senior lecturer at Western Sydney University, will contribute to data interpretation and manuscript preparation in his role as co-investigator.

Brice, K. L., Trivedi P., Jeffries T. C., **Blyton M. D. J.**, Mitchell C., Singh B. K., & **Moore B. D.** (2019) The Koala (Phascolarctos cinereus) faecal microbiome differs with diet in a wild population. *PeerJ*. 7, p.e6534

Blyton M.D.J, Soo R.M., Whisson D., Marsh K.J., Pascoe J., Le Pla M., Foley W., Hugenholtz P. & **Moore B. D.** (2019) Faecal inoculations alter the gastrointestinal microbiome and allow dietary expansion in a wild specialist herbivore, the koala. *Animal Microbiome*. DOI: 10.1186/s42523-019-0008-0

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