KOALA HEALTH HUB

Collecting koala scats in the field

KOALA HEALTH PROTOCOL

Introduction

Scats are increasingly being used to obtain non-invasive samples, allowing relatively inexpensive access, or access to material from cryptic populations. Koala scat surveys focusing on animal abundance and habitat distribution have been used widely (Lunney et al. 2000; Sullivan et al. 2004; McAlpine et al. 2006); however, the use of koala scats for koala disease surveys is relatively new and gaining interest as a non-invasive alternative method that avoids the costs and risks associated with koala capture (Wedrowicz et al. 2013; Wedrowicz et al. 2016). It provides the only form of genetic and disease data collection from some cryptic koala populations and those populations found in terrain too inaccessible or difficult for koala capture studies (Wedrowicz et al. 2016; Wedrowicz et al. 2017; Wedrowicz et al. 2019; Schultz et al. 2018).

Uses include:

- extraction of DNA for use in host genetics and pathogen detection studies
- analysis of faecal stress or reproductive hormones.

There are significant limitations to the use of scats and understanding these is essential to study success.

Study design considerations

Pathogen detection

Scats can be used to detect some pathogens (agents of disease), i.e. those that are shed into the intestinal tract or anal-urogenital opening. While they are better than nothing, there are several considerations in sample design:

Sampling strategy is important – Detecting and estimating prevalence of infection or freedom from infection in a population is a statistical exercise requiring unbiased sampling of a sufficient sample size. Proper design requires data on population size, clustering, expected prevalence, required degree of certainty and confidence intervals, and sensitivity and specificity of the assay. Design may vary depending on the question being asked and the specific context of the study. Further work is needed to develop models to better predict prevalence and associated confidence intervals from small data sets from samples tested with variable sensitivity. Veterinary epidemiological input is essential to effective study design.

Infection does not equal disease – while agents of disease may be detected in scats, this often says nothing of the health status of the population, as infected animals may or may not be diseased. This is particularly true of agents infecting koalas, such as chlamydia and koala retroviruses, for which infection without disease is common – especially in more resilient populations. For any conclusions to be drawn on disease, animal capture and clinical examination is required. Low quality data on disease may be collected from observation of sighted animals (coat quality, ocular disease, rump staining, absence of back young when expected) but will likely underestimate true levels.

Shedding of pathogen – Chlamydia and KoRVA are reliably detected in fresh, high quality scats but scats have not yet been validated for detection of other agents such as KoRV subtypes (B–J). Other less known agents such as herpes viruses and Trypanosomes are unlikely to be detected by this route.

Sensitivity and impact on sample size and cost – The age of koala scats, exposure to weather conditions and conditions of storage all have been shown to significantly affect assay sensitivity and interpretation of results. In order to preserve DNA in epithelial cells on koala faecal pellets the following should be avoided: handling and contact with other surfaces, exposure to UV light, repeated freezing and thawing, and bacterial growth (i.e. warmth or moisture).

- The detection of *Chlamydia pecorum* by polymerase chain reaction (PCR) from swab DNA samples is considered as the gold standard (Hanger et al. 2013). On average, amounts of *C. pecorum* DNA were 3000 times greater in DNA from swabs, compared to that of scat DNA (Wedrowicz et al. 2016).
- *C. pecorum* can be detected from fresh koala scat DNA with >80% concordance relative to swabs (Wedrowicz et al. 2016; Koala Health Hub 2017; OWAD Environment 2017).
- Sensitivity of detection is reduced by DNA degradation and presence of PCR inhibitors. Testing must be accompanied by a test for DNA quality.
- Storage conditions are important – PCR amplification rates can drop from 62% after one week of storage down to 1% after four weeks (Wedrowicz et al. 2013), or can remain stable (90%) for at least four weeks under good conditions.

More in-depth evaluation of the effect of collection, storage and DNA extraction methods on DNA quality of fresh and aged scats is required. In the meantime, the recommendation for the use of scat samples in genetic and disease studies should be that samples be as fresh as possible and handled according to strict protocols to avoid DNA degradation, removal of DNA from the scat surface, or cross-contamination.

Quantity and quality of scat DNA must be assessed in the laboratory to reject samples for which assay sensitivity will be compromised (Koala Health Hub 2017; OWAD Environment 2017, p. 14; Wedrowicz et al. 2017; Wedrowicz et al. 2018). A significant number of samples will need to be collected, extracted and assessed and then rejected, with impacts on sample size and cost of processing. As a rule of thumb, 50% should be added to sample size and associated collection and laboratory costs, but this will vary with scat age and quality.

Sample duplication (unknowingly sampling the same animal twice) can skew data – While distance and time can be used to deduce independence of sampling, genotyping is more definitive and less restrictive on sampling design. However, identification is based on analysis of 12 microsatellites, which can be challenging with degraded DNA. Even for fresh koala scats complete genotypes were obtained for only 66–75% of samples (Wedrowicz et al. 2017; OWAD Environment 2017) and much less for aged samples from cryptic populations (Koala Health Hub 2017).

Analysis of faecal stress or reproductive hormones

The assay of scats for excreted stress hormones or their metabolites is used worldwide as an indicator of stress to individuals or populations. Its use in koalas has been fairly limited to date and requires further validation to determine whether observed changes are within normal homeostatic limits or are indicative of host compromise. If validated, they would comprise a potentially useful complement to other means of health and immune assessment. Pellets must be collected very fresh (i.e. still moist) and then frozen within 24 hours.

Host genetics

Reliable genetic information (sex and genotyping) can be obtained by extracting DNA from koala scats (Wedrowicz et al. 2013; Wedrowicz et al. 2016; Schultz et al. 2018) but due to DNA degradation, it is suitable for only some genetic methods. For any method, some failure of samples should be anticipated. As already stated, even for fresh koala scats complete genotypes were obtained for only 66–75% of samples (Wedrowicz et al. 2017; OWAD Environment 2017). Geneticists should be consulted during study design.

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Scat sampling protocol

Assess the pellet

a

b

c

d

Figure 1: Scat assessment: a) Category 1 fresh scat (covered in mucus, wet or dry and shiny); b) Category 2 medium fresh scat (no shine but smells when broken); c) Category 3 old scat (no shine, no smell when broken, may be some disintegration); d) scats of different sizes from different individuals (Cristescu & Frere 2017)

Collect the pellet

Metadata

The value of scats is dramatically increased by collection of comprehensive metadata. See recording sheet below.

Pathogen detection

- Avoid handling the surface of the pellet as much as possible. Pathogens of interest such as chlamydia will be more concentrated on the outside surface of the scat pellet.

The following methods have been used with success but have not been evaluated against each other to determine which is best:

Buffer method

- Place pellets into a screw-cap tube containing 5 mL lysis buffer (100mM Tris-HCL, 50mM EDTA, 1% w/v SDS, pH 8; Koala Health Hub, University of Sydney, damien.higgins@sydney.edu.au).
- Label and store at room temperature or refrigerate. Submit to the laboratory for processing within two weeks (pers. comm., A Casteriano, Koala Health Hub, University of Sydney).

Or

Freezing method

- Place the scats into a plastic tube, label the tube and place immediately onto ice. At the end of the day, transfer to the freezer. Avoid freeze–thaw cycles during shipping and storage (Cristescu & Frere 2017).

Or

Drying method

- Very gently scoop up the scats using a ziplock bag or paper envelope. Avoid squashing the pellets and do not collect so many that they pile up on top of each other, up to 10 pellets is a good number for a sandwich-sized ziplock bag.
- Leave the mouth of the bag stretched wide open, in a dry environment. Gently shake the bag to rotate the scats every few hours during the 1st day, then two or three times a day after that. This prevents any single surface of a scat from staying in contact with the plastic bag.
- If moisture builds up in the bag, divide the scats into another bag to allow more room between them, and ensure the environment is dry and the bag well opened.
- Allow the scats to dry completely and develop a hard and dry coating on the outside. This could take up to three days depending on the weather conditions.
- Once dry, for storage and submission to the laboratory, gently empty the scats into a small paper envelope and put this inside another envelope for extra protection, or place them into a 30 mL plastic specimen jar with a sachet of desiccant (silica gel).
- Submit to the laboratory (pers. comm., K Leigh, Science for Wildlife).

Faecal stress or reproductive hormones

Collect fresh scats (Category 1) into a plastic container, keep cool, and freeze within 24 hours.

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Koala scat recording sheet

General location:			Datum for grid refs:			Other comments:			
ID	**Date**	**Easting**	**Northing**	**Number of pellets**	**Size L x W (mm)**	**Pellet condition (1–3)**	**Collection method**	**Animal observations: Tag, eyes, rump, fur, sex, age, other, not seen**	**Tree species**