Supplementary material

DNA isolation method used for 2007 samples

Excerpt from Karsa, M.M. (2007) Genetic analysis of koala populations using DNA extracted from faecal material. Honours Thesis, Monash University, Churchill

Intestinal epithelial cells on the faecal pellets were collected by placing individual pellets in sterile screwtop vials (Cospak Pty. Ltd.) with 800 – 2000 µl phosphate- buffered saline (50 mM $NaH_2PO_4.2H2O$, 50 mM Na_2HPO_4, 0.15 M NaCl, pH 7.4), sufficient to allow recovery of approximately 400 µl following the wash. The surface of the pellet was washed gently by rolling the vials on the Gyratory Mixer (Ratek Instruments Pty. Ltd.) for eight minutes. 200 µl of the wash was removed and placed in a 2 ml-microcentrifuge tube. Another 200 µl of the wash was also removed and transferred into a second 2 ml-microcentrifuge tube.

DNA was extracted from the recovered epithelial cells using the QIAamp® DNA Stool Mini Kit (Qiagen Pty. Ltd.). Cells were lysed by the addition of 1.6 ml of Buffer ASL to each 200 µl extract, followed by incubation at 35ºC for one hour. Samples were vortexed occasionally (once every 15-30 minutes) to ensure samples were thoroughly mixed. Samples were then centrifuged at 13000 x g (13200 rpm) for 1 minute to pellet the debris.

The supernatant (around 1.4 ml) was pipetted into a new 2 ml-microcentrifuge tube. One InhibitEX™ tablet was added to each sample to remove inhibitory materials that might be present. Samples were vortexed until the tablet dissolved, then incubated at room temperature for one minute to allow inhibitors to adsorb to the InhibitEX™ matrix. Samples were then centrifuged at 13000 x g (13200 rpm) for 25 minutes to pellet inhibitors bound to the InhibitEX™ matrix.

The supernatant (around 600 µl) was immediately removed into 2 ml-microcentrifuge tubes followed by addition of 20 µl of 20 mg/ml Proteinase K. 600 µl of Buffer AL was added to each sample; a homogenous solution was achieved by vortex mixing. The mixture was then incubated at 70ºC for 10 minutes. DNA was precipitated by the addition of 600 µl 99.9% ethanol to the lysate.

Around 680 µl of the first lysate from each pellet was pipetted onto QIAamp® spin columns before centrifuging at 13000 x g (13200 rpm) for one minute. The filtrate was discarded. This step was repeated so that both extracts from each pellet passed through a single column.

500 µl of washing buffer, Buffer AW1 was added onto the QIAamp® spin column before centrifuging at 13000 x g (13200 rpm) for one minute. Collection tubes containing the filtrate were discarded. The QIAamp® spin columns were then placed into new collection tubes. 500 µl of washing buffer, Buffer AW2 was added onto the columns before centrifuging at 13000 x g (13200 rpm) for 4 minutes. Centrifugation time was increased to ensure smooth downstream applications as residual Buffer AW2 in the eluate may inhibit subsequent PCR (QIAamp® DNA Stool Mini Kit Handbook, 2001). Collection tubes containing the flow-through were discarded.

After placing the QIAamp® spin columns into fresh, 2-ml microcentrifuge tubes, 100 µl of Buffer AE was pipetted directly onto the QIAamp® membrane. Columns were incubated at room temperature for five minutes, and then centrifuged at 13200 rpm for one minute to elute DNA. The elution step was repeated with another 100 µl of Buffer AE to increase DNA yield. The extracted DNA was stored at 4ºC.

Table S1. Number of loci positively amplified and scored, and the proportion of available loci that were identical, in samples genotyped in duplicate. Molly and Marlo were sampled in both 2007 and 2013. All other duplicates were sampled in 2013.

Koala name	Positive loci	Identical loci
Molly (2013)	12	100%
Molly (2007)	11	
Marlo (2013)	12	100%
Marlo (2007)	8	
Mitta	12	100%
Mitta	12	
Mitta	12	
Lisa	12	100%
Lisa	11	
Lara	12	100%
Lara	12	
Lennox	10	100%
Lennox	10	
Han	12	100%
Han	11	
Vivian	12	100%
Vivian	12	
Bernie	12	100%
Bernie	12	

Table S2. Summary of putative parent-offspring displaying incompatible genotypes. Potential parents identified, based on the absence of any mismatching loci.

Parent	Offspring	Mismatched loci	Potential parent/s
Marrguk ♀	Lorien ♀	3	None identified
Nellie ♀	Kevin ♂	4	Jupiter ♀
Banjo ♂	Lisa ♀	6	Ganymede ♂
Vivian ♂	Lennox ♀	4	Mantis ♂, Nautilus ♂
Merv ♂	Lara ♀	1	Banjo ♂

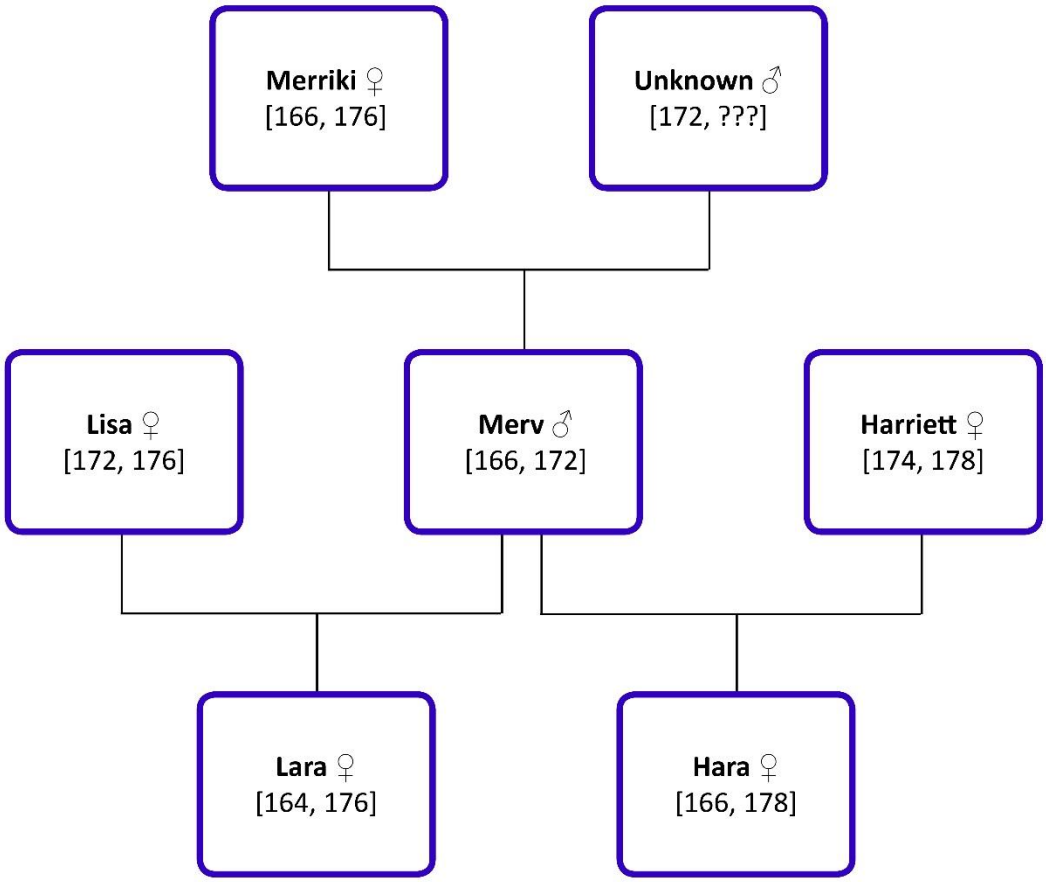

Fig. S1. Pedigree for locus K2.1 where incompatible alleles were found between Merv and Lara. The 166 bp allele is not likely to be an error as it is present at K2.1 of his mother (Merriki) and another of his offspring (Hara). Two identical consensus genotypes were obtained independently for Lara. The 164 bp allele at K2.1 was present in an additional seven KCC individuals. The 164 bp allele is also not likely to be an error. Given Lisa as the known mother of Lara, CERVUS identified Banjo as the most likely father of Lara with a significant odds ratio obtained for the trio (Lara−Lisa−Banjo). Further analysis using additional markers may be required to confidently identify paternity for Lara.