Animal (2010), **4:2**, pp 282–288 © The Animal Consortium 2009
doi:10.1017/S1751731109991078

animal

Using faecal DNA to determine consumption by kangaroos of plants considered palatable to sheep

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(Received 11 December 2008; Accepted 14 September 2009; First published online 20 October 2009)

*Disagreement exists within the scientific community with regards to the level of competition for feed between sheep and kangaroos in the Australian rangelands. The greatest challenge to solving this debate is finding effective means of determining the composition of the diets of these potential grazing competitors. An option is to adopt a non-invasive approach that combines faecal collection and molecular techniques that focus on faecal DNA as the primary source of dietary information. As proof-of-concept, we show that a DNA reference data bank on plant species can be established. This DNA reference data bank was then used as a library to identify plant species in kangaroo faeces collected in the southern rangelands of Western Australia. To enhance the method development and to begin the investigation of competitive grazing between sheep and kangaroos, 16 plant species known to be palatable to sheep were initially targeted for collection. To ensure that only plant sequences were studied, PCR amplification was performed using a universal primer pair previously shown to be specific to the chloroplast transfer RNA leucine (*trn*L) UAA gene intron. Overall, genus-specific, single and differently sized amplicons were reliably and reproducibly generated; enabling the differentiation of reference plants by PCR product length heterogeneity. However, there were a few plants that could not be clearly differentiated on the basis of size alone. This prompted the adoption of a post-PCR step that enabled further differentiation according to base sequence variation. Restriction endonucleases make sequence-specific cleavages on DNA to produce discrete and reproducible fragments having unique sizes and base compositions. Their availability, affordability and simplicity-of-use put restriction enzyme sequence (RES) profiling as a logical post-PCR step for confirming plant species identity. We demonstrate that PCR–RES profiling of plant and faecal matter is useful for the identification of plants included in the diet of kangaroos. The limitations, potential and the opportunities created for researchers interested in investigating the diet of competing herbivores in the rangelands are discussed.*

Keywords: faecal DNA, dietary components, kangaroos, rangelands

Implications

The ability to positively identify components in the diet of grazing animals via faecal analysis is the first step to enabling determination of the degree of competition between different grazers within any one environment. Ultimately, this information will enable livestock managers to make informed decisions regarding sustainable grazing management practices, including the control of native and feral animals that are competing with livestock for limited feed resources.

Introduction

The southern rangelands of Western Australia are a low-rainfall area covering 600 000 km^2 of arid to semi-arid shrublands (Mitchell *et al*., 1994). The area experiences a dry climate with hot summers and cool winters. The average, annual rainfall is below 300 mm and usually falls in winter; however, this often varies dramatically from year to year especially with summer cyclones. Vegetation is dominated by native species, being a variable mix of annuals and perennials depending on location and seasonal conditions. Pastoral properties rely on the production of stock grazing the native vegetation in the rangelands. Economic success in the long term relies on the manager not degrading the vegetation base that supports production, but there are no established methods for easily identifying which plants are being grazed by stock (both domestic and native) in the rangelands. Kangaroo (red – *Macropus rufus*, and western grey – *Macropus fuliginosus*) numbers may be as high as four to five kangaroos per km^2 (Southwell *et al*., 1991; Norbury *et al*., 1993), whereas sheep densities are in the

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range of 2 to 13 dry sheep equivalents (DSE) per km^2 (Pringle *et al*., 1994). Pastoralists consider these kangaroos as competitors of sheep, although disagreement exists within the scientific community with regards to the level of competition for feed between sheep and kangaroos. The greatest challenge to solving this debate is finding a non-invasive and effective means of determining the actual composition of the diets of these potential grazing competitors.

Determining the botanical composition of the diet of both sheep and kangaroos (and other macropods) has relied predominantly on field observation (Short, 1986), examination of stomach contents (Taylor, 1983; Dawson and Ellis, 1996; Sprent and McArthur, 2002) or epidermal tissue trace analyses of faecal material (Wann and Bell, 1997; Holechek *et al*., 2004). All of these techniques are either invasive or time consuming and of little practical use in identifying the diet composition of animals grazing on the complex and (plant) species-rich rangeland ecosystems.

Another potential 'marker' for determining diet composition of herbivores is saturated hydrocarbons or alkanes, which are found in the cuticular wax of plants (Dove and Mayes, 2005). Determining diet composition from plant alkanes works best in a temperate, sown pasture containing a low number of different plant species. Furthermore, validations of the alkane method have mainly been confined to grasses or grass–legume associations (Valiente *et al*., 2003). In the rangelands, browse may constitute a large proportion of the feed available to grazing animals and the validity of the alkane method for such plants has not been done. The use of alkanes is expensive and the procedure is difficult in heterogenous pastures (Lee and MacGregor, 2004). Some of the issues associated with using these markers for determining diet composition include (i) alkanes can differ between stem, leaf and flowerhead and seasons (Smith *et al*., 2001); (ii) alkanes may vary with the ages of some plants (Lee and MacGregor, 2004); and (iii) errors in sampling and chemical analysis can yield negative answers (Newman *et al*., 1995).

Molecular faecal analysis is a potential non-invasive alternative and the need for a greater level of discrimination has seen the recent increase in adoption of DNA-based technology, particularly PCR amplification. The exquisite sensitivity and specificity afforded by PCR are particularly useful under conditions where the target is present at low copy number and in a potentially non-permissive matrix (Saiki *et al*., 1985; Zhao *et al*., 2002). Recently, faecal DNA amplification has been used for the evaluation of diets in sea lions (Deagle *et al*., 2005) and of marine vertebrate predators (Jarman *et al*., 2002). In this study, faecal DNA was used as a means of determining dietary components of kangaroos grazing on the southern rangelands of Western Australia.

Material and methods

Study site

The field study was conducted between June 2006 and November 2007 on 'Mendleyarri Station' (121°40′E, 29°46′S), a large pastoral station in the southern rangelands of Western Australia. Mean annual rainfall is 249.6 mm and rainfall is highly variable. The area is dominated by native vegetation, consisting of variable mix of annual and perennial grasses and browse species. Sheep grazing was the primary land use.

The two paddocks chosen for this study were part of a larger study on diet selection in sheep and the impact of drought on such selection. The larger of the paddocks (10 200 ha) had not been grazed by sheep for more than 5 years, during which time plant species considered desirable for sheep grazing had become re-established in the land system. The second paddock (3700 ha) had been permanently stocked with Damara sheep for at least 3 years. Continuous overgrazing of this paddock had led to a general loss of desirable, palatable and perennial plant species.

In both paddocks, fixed dung collection sites (8.5 × 5.0 m) were installed at approximately 1 km intervals along a transect running east and west from the central water point. Four dung collection sites were installed in the unstocked paddock and seven in the stocked paddock. The sites were also part of a larger study (S. van Wyngaarden unpublished data) on the impact of distance from water on grazing pressure, with grazing density determined using the area of the collection site, the dry matter weight of faeces collection and the number of days over which the faeces accumulated.

Plant selection

The plant species targeted for collection (Table 1) for DNA were known to be palatable to sheep based on pastoralists' observations of sheep grazing, evidence of utilisation observed in the field and information reported in Mitchell *et al*. (1994) and Russell and Fletcher (2003). Identification of the plant species was confirmed by Associate Professor Ben Norton (Curtin University of Technology) using the internet programme Flora Base (Western Australia Conservation and Land Management, 2008). Vouchers of the targeted plants have also been deposited with the Western Australian herbarium. Within 8 h of collection in the field, the samples were dried over 2 days at 60°C in a fan-forced oven, before being transported to the ChemCentre for subsequent analysis.

Faecal collection

Recent (retained, intact surface and some patina, therefore less than 2 days old) faecal samples were intermittently collected from the dung collection sites. Kangaroo faeces were readily distinguished from those of ruminants. Within 8 h of collection, the faecal samples were dried over 2 days at 60°C in a fan-forced oven, before being transported to the ChemCentre for subsequent analysis. A total of 63 samples were collected from the collection sites during the study period.

DNA analyses

The first requirement was the establishment of a DNA reference data bank for the selected plant species. DNA was isolated from the plants' chloroplast and subjected to PCR

Table 1 *Rangeland plant species referenced in this study and their PCR amplification using primer pairs to the chloroplast genome*

Scientific name	Common name	CH53/55 (bp)	CH 37/39 (bp)
Grasses:			
Aristida contorta F. Muell.	Wind grass	616	800
Austrostipa elegantissima Labill	Silver speargrass	602	802
Enneapogon caerulescens (Gaudich)	Limestone grass	635	804
Browse:			
Acacia hemiteles Benth.	Tan wattle	637	1360
Atriplex bunburyana F. Muell.	Silver saltbush	489	774
Atriplex vesicaria Benth.	Bladder saltbush	492	772
Eremophila forrestii F. Meull.	Wilcox bush	557	799 & 280
Eremophila maculata subsp. *brevifolia* (Ker Gawl.) F. Muell.	Emu bush	570	802
Frankenia setosa L	Frankenia	530	789
Maireana georgei (Diels) Paul G. Wilson	George's bluebush	660	778
Maireana pyramidata (Benth.) Paul G. Wilson	Sago bush	660	781
Maireana sedifolia (F. Muell.) Paul G. Wilson	Pearl bluebush	647	781
Ptilotus obovartus (Gaudich.) F. Muell.	Cotton bush	725	773
Rhagodia eremaea Paul G. Wilson	Tall saltbush	640	769
Scaevola spinescens R. Br.	Current bush	554	760
Solanum lasiophyllum Poir	Flannel bush	577	802

amplification (as described below). DNA restriction site screening was then performed on respective PCR-amplified DNA to which the resultant restriction profiles were used to assign the reference plants. Using the same procedures, DNA was isolated from the faecal samples and PCR-amplified using the same primer pairs as used for the reference plant material.

Isolation of DNA. The dried faecal samples were individually milled, sieved through a 0.5 mm in a Retsch Model SK-100 (1100 W) cross-beater mill. Each sample was decanted into a plastic sample bottle, labelled and stored at room temperature until subsequently used for DNA analysis. To further minimise the risk of cross-over contamination, the entire mill was thoroughly cleaned in-between samples using compressed air and then wiped down with tissues.

Genomic DNA was isolated from milled faecal samples using the QIAGEN DNeasy Plant kit (QIAGEN, Hilden, Dusseldorf, Germany), with modifications. Each sample was analysed in triplicate. The 25 mg subsample was combined with 1.2 ml of Lysis buffer (Buffer AP1) and 12 μl RNase A stock solution (100 mg/ml). The mixture was incubated for 10 min at 65°C after which 390 μl of Buffer AP2 was added, mixed and the lysate placed on ice for 5 min. The lysate was centrifuged for 5 min at 20 000 × **g** and 560 μl from the supernatant was used for DNA isolation according to the manufacturer's instructions, without alteration.

For the reference plants, dried leaves were placed in a plastic bag and manually ground into smaller fragments using a bottle as a rolling pin. The three 25 mg samples were taken for DNA isolation using the QIAGEN DNeasy Plant kit. A new bag was used for each reference plant. All genomic DNA were eluted to a final volume of 100 μl AE Buffer (QIAGEN) and stored at −20°C.

PCR amplification. DNA was screened for suitability for PCR by amplifying each isolate over a twofold serial dilution range. The best performing range of titres for each DNA preparation were those which produced a single and most intensely stained DNA band (DNA concentration ranging from 5 to 25 ng was optimal for PCR amplification), and these were used for subsequent studies. PCR amplifications were performed on 5.0 μl of DNA isolate in a final reaction volume of 20 μl containing 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl (pH 8.3), 0.25 μM primers, 250 μM dNTPs and 0.75 U Kapa Taq DNA polymerase (Kapa Biosystems, Observatory, Cape Town, South Africa). The primer pairs used were CH 37/39 (*trn*L and *rpl*2m1) and CH 53/55 (*trn*L exons 1 and 2; see Table 2). DNA was denatured at 94°C for 2 min, followed by seven cycles of 94°C for 15 s (denaturation) and 72°C for 1 min (extension), in which the annealing temperature was touch downed in 1° decrements, from 65°C to 58°C. This was followed by 28 to 30 cycles of amplification in which the annealing temperature was held at 58°C and before a final extension of 72°C for 10 min. The presence of PCR-amplified products were confirmed by UV transillumination following horizontal gel electrophoresis on a 2% agarose (Bio-Rad, Hercules, CA, USA) and then accurately sized using the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions.

Restriction enzyme analysis. Up to 10 μl of amplified DNA (250 to 500 ng) was digested with restriction enzymes (Roche Diagnostics, Indianapolis, IN, USA) in a final volume of 18 μl for 4 h at 37°C. A panel of 24 different restriction enzymes were used to generate restriction profiles from which DNA fingerprints diagnostic for each plant species were identified. The presence of restriction fragments was confirmed by UV transillumination following horizontal gel electrophoresis on

Table 2 *Primer pairs used*

Primer	Sequence 5′ to 3′ (priming region)	Reference
CH 37 F	TGA ATG GTT AAA GCG CCC AAC T (*trn*L)	Heinze (2007)
CH 39 R	TTC TAT GGT TAC GAT TCT ACC ATA TAT GTC (*rpl*2m1)	Heinze (2007)
CH 53 F	CGA AAT CGG TAG ACG CTA CG (*trn*L exon 1)	Taberlet *et al.* (1991)
CH 55 R	GGG GAT AGA GGG ACT TGA AC (*trn*L exon 2)	Taberlet *et al.* (1991)

F=forward primer; R=reverse primer.

4% agarose MS (Roche Diagnostics), and the fragments were accurately sized using the Agilent 2100 bioanalyzer (Agilent Technologies) according to the manufacturer's protocol.

Results

PCR amplification of target plant species

DNA fragments of varying sizes were obtained when the *trn*L intron was amplified in DNA isolates from a range of browse and grass species. The results of PCR amplification are summarised in Table 1. The limit of resolution appeared to be in the order of 5 bp as exemplified in *Atriplex vesicaria* and *Atriplex bunburyana.* It can be seen that the CH 53/55 primer pair was able to size-differentiate all of the species except for *Maireana georgei* and *Maireana pyramidata*, which both migrated at 660 bp. Amplification with the CH 37/39 primer pair produced monospecific amplicons, with the exception of *Eremophila forrestii* that presented a major band at 280 bp and a minor band at 799 bp. The largest recorded amplified product was *Acacia hemiteles* (1360 bp). Plant species that could not be differentiated with CH 37/39 were *Solanum lasiophyllum*, *Eremophila maculata* and *Austrostipa elegantissima,* which each presented a 802-bp PCR product and *Maireana sedifolia* and *M. pyramidata,* which both presented a 781-bp product. For these reasons, the CH 37/39 primer pair was not considered further. Overall, (plant) species prediction on the basis of PCR length heterogeneity alone was more successful with CH 53/55 than with the CH 37/39 primer pair.

Restriction enzyme analysis

An array of DNA fragment length polymorphisms was generated by digestion with a panel of 24 restriction endonucleases, enabling the selection of DNA fingerprints, which were diagnostic for each plant species (data not shown). The restriction enzyme site profile of CH 53/55 amplified DNA on representative browse species are shown in Table 3. The presence of *Mva 1* and *Nde II* restriction sites clearly distinguished *A. hemiteles* from *Rhagodia eremaea*. The *Rsa 1* restriction sequence appeared to be unique only to *E. forrestii*. Two enzymes, *Eco R1* and *Ssp 1* could be used to differentiate between *A. vesicaria* and the closely related *A. bunburyana*. However, *M. georgei* and *M. pyramidata* could not be differentiated.

Diet composition as determined from PCR amplification of kangaroo faeces

DNA isolates from kangaroo faeces were extensively degraded. Nevertheless, the presence of high molecular weight fragments inferred their suitability for PCR amplification. Of the 63 faecal samples subjected to DNA analysis, seven were PCR negative. Five of the amplified 'bands' from the faecal samples did not conclusively match any of the reference plant species.

As shown in Table 4, of the plant species selected for this study, only *M. sedifolia* was not consumed at some stage by the kangaroos under study. Detectable differences in the molecular weights (expressed in bp) of *A. bunburyana* and *A. vesicaria* were possible with the plant samples; however, in the faecal samples, when compared against the reference plants' DNA molecular weight ladder, it was not possible to clearly differentiate which Atriplex was being consumed. The same situation arose at times with differentiating between *E. forrestii* and *E. maculata* and also between *Enneapogon caerulescens* and *R. eremaea* in the faecal samples.

Kangaroos consumed both grasses and browses throughout the study. Of the grasses, *Aristida contorta* consistently appeared in the faeces, and of the browse species *S. lasiophyllum* was also consistently consumed except for the late spring/early summer of 2007. The consumption of browses appeared to be greater (or more diverse) during autumn and winter compared to spring and early summer.

Discussion

DNA barcoding of rangeland plants

The aim of this investigation was to assess the effectiveness of molecular genetic techniques at discriminating between plant species collected from the southern rangelands of Western Australia and then using this same technology on faecal samples collected in the field to determine dietary components of kangaroos grazing on these rangelands.

The *trn*L non-coding chloroplast sequences of plant species were PCR amplifiable from DNA isolates from both plant and faecal samples. There was sufficient sequence divergence to enable a differentiation at the taxon level between the browse species, although with exceptions.

The critical issue has been the judicious selection of unique priming sequences that serve as markers specific for particular plant taxons. The chloroplast transfer RNA leucine (*trn*L) UAA gene intron is a non-coding region that possesses sufficient sequence variability enabling greater phylogenetic utility on plant species identification (Taberlet *et al.*, 1991; Gielly and Taberlet, 1994) and when combined with restriction enzyme digestion provides a further level of resolution at the genus/species level (Kojoma *et al.*, 2002;

Table 3 *Restriction enzyme site profile of CH 53/55 amplified DNA of representative browse species from the southern rangelands of Western Australia*

Restriction enzyme	*A. hemi*	*R. erem*	*P. obov*	*S. lasi*	*E. forre*	*M. geor*	*M. pyra*	*A. vesic*	*A. bunb*	*E. caer*
Alu 1	+	+	+	+		+	+	+	+	+
Asp 1	+	+	+	+		+	+	+	+	+
Bam H1										
Ban II										
Bgl 1										
Cfo 1										
Cla 1		+				+	+		+	
Dde 1	+	+	+	+	+	+	+	+	+	+
Dra 1				+	+	+	+		+	+
Eco R1		+	+		+			+		
Hae III					+					+
Hind III					+					
Hinf 1	+	+	+	+		+	+	+	+	+
Hpa II				+	+			+	+	+
Mva 1	+		+	+	+	+	+	+	+	
Nde II	+		+	+	+	+	+	+	+	
Nsi 1										
Pst 1										
Rsa 1					+					
Sal 1		+	+	+	+	+	+	+	+	+
Sau 3A1	+	+	+	+		+	+	+	+	
Ssp 1		+	+	+		+	+		+	+
Taq 1		+	+	+		+	+	+	+	+
Xba 1		+								

A. hemi = *Acacia hemiteles*; *R. erem* = *Rhagodia eremaea*; *P. obov* = *Ptilotus obovartus*; *S. lasi* = *Solanum lasiophyllum*; *E. forre* = *Eremophila forrestii*; *M. geor* = *Maireana georgei*; *M. pyra* = *Maireana pyramidata*; *A. vesic* = *Atriplex vesicaria*; *A. bunb* = *Atriplex bunburyana*; *E. caer* = *Enneapogon caerulescens*.
+ denotes presence of restriction enzyme site.

Table 4 *Plant species identified in the faeces of kangaroos collected from the four collection sites*

Date	Plant species identified by DNA
June 2006 ($n = 7$)	*A. hemiteles*, *A. contorta*, *Atriplex* spp., *A. elegantissima*, *Eremophila* spp. (*forresttii* or *maculata*), *Frankenia setosa*, *Mairaena* spp. (*georgei* or *pyramidata*), *P. obovatus*, *S. spinescens*, *S. lasiophyllum* (*E. caerulescens* or *R. eremaea*)
April 2007 ($n = 10$)	*A. hemiteles*, *A. contorta*, *Atriplex* spp., *E. maculata*, *Frankenia setosa*, *Mairaena*spp. (*georgei* or *pyramidata*), *S. lasiophyllum*, (*E. caerulescens* or *R. eremaea*)
June 2007 ($n = 19$)	*A. contorta*, *A. elegantissima*, *E. maculata*, *Frankenia setosa*, *S. lasiophyllum*, *S. spinescens* (*E. caerulescens* or *R. eremaea*)
September 2007 ($n = 16$)	*A. hemiteles*, *A. contorta*, *A. elegantissima*, *E. maculata*, *S. lasiophyllum*
November 2007 ($n = 11$)	*A. hemiteles*, *A. contorta*, *E. forrestii*

Ridgway *et al*., 2003). Compared to the CH 37/39 primer pair (Heinze, 2007), the CH 53/55 primer pair produced diagnostic bands of greater size variability that improved resolution for differentiation between the plant species.

Because there is the possibility that plant species other than those used here could be co-amplified, it prompted the adoption of validation by restriction enzyme site profiling. Generally, there was sufficient sequence divergence between the browse species to generate the required restriction fragment length polymorphisms. However, it was not possible to differentiate between *M. georgei* and *M. pyramidata* with the system used in this study. In this case, it appears that the *trn*L region did not possess the appropriate sequence polymorphism for differentiation. This could be resolved by screening other primer pairs that are accessible from current databases especially for those that are specific for the chloroplast genome (Heinze, 2007). Nevertheless, the presence or absence of particular plant species can be resolved by restriction enzyme digestion provided that 'informative' enzymes are used. It would seem from this model that, more than one primer pair would be required to correctly identify individual plant species.

The adoption of molecular techniques, for example, PCR amplification and restriction enzyme fragment profiling, further strengthens the evaluation process by enabling the accurate identification of forages species. A logical

progression would be the extension of the current browse collection and discover other suitable primer pairs that enable species determination by PCR length heterogeneity.

PCR amplification of kangaroo faeces

The enormous heterogeneity of sequences presented by faecal DNA required the adoption of a priming system that excluded all except those of plant species origin. Furthermore, suitable markers were needed to accommodate the extensive degradation and sequence diversity expected in DNA isolates from faeces. A chloroplast-based PCR approach was chosen as photosynthesis is a vital survival mechanism in angiosperms; chloroplast DNA is more abundant than nuclear DNA (Timmins, 2003); and access to sequence information from Genbank or equivalent databases (Taberlet *et al*., 1991; Gielly and Taberlet, 1994) increases the prospect of finding other suitable primers. Although priming domains chosen were reported to be highly conserved across plant taxa, the risk from using such primers is that non-target contaminants of plant origin could be amplified unintentionally. Prevention of such risks included the monitoring of PCR reagent blanks that are prepared under DNA-free conditions.

Specific sequences on the chloroplast genome can be PCR-amplified from a faecal DNA matrix, but at lower efficiency. That this constraint was also an effect from extremely low levels of homologous sequences was consistent with the spontaneous increase in intensity of PCR-amplified bands following spiking with plant DNA (data not shown). There are risks associated with amplification of low copy number sequences including the potential for compromising the process of plant residue identification. Therefore, we minimised such risks by adopting strategies that included: (i) monitoring carryover contamination with PCR reagent blanks; (ii) eliminating contamination from pipettors by using filtered tips; (iii) confirming identity of amplicons by size with reference to plant standards; [iv] optimising the number of amplification cycles to minimise non-specificity and to maximise sensitivity; (v) maintaining a high level of stringency by using the highest permissible annealing temperature; and (vi) using a touchdown programme to select for homologous sequences to those of the primers.

The ability to estimate relative proportions of plants in the diet would provide useful information on issues such as plant preference and palatability. Information on chloroplast content in each feed species (on the copies per cell basis) and other factors that contribute to 'PCR bias' are needed to standardise proceedings for quantification. In the context of this project, all PCR runs were performed to the endpoint to maximise amplification productivity, which is not quantitative. The risk of misrepresentation can be minimised by performing PCR amplification under 'real-time' conditions, which is the subject of a follow-up study.

Although species identification can be performed according to amplicon size alone, there is the possibility that plant species other than those used here could be represented by DNA bands on an agarose gel. This potential for misidentification prompted the adoption of another tier of validation in restriction endonuclease digestion. Moreover, there was sufficient sequence divergence to generate the required restriction fragment length polymorphisms. A set of restriction enzymes were identified that offered reliable identification of plants based on the presence or absence of cleavage sites. This library of genetic signatures simplified the species identification of plant residue, which is particularly advantageous for laboratories that do not yet have access to DNA sequencing or other equivalent resources.

Factors residing in the kangaroo faecal matrix could fortuitously co-purify with DNA and inhibit PCR amplification. It is worth exploring other PCR priming systems that are reported to amplify across highly polymorphic regions on the chloroplast genome (Taberlet *et al*., 1991; Timmins, 2003). Species-specific primers to complement the 'universal' primers should also be considered. This could increase the likelihood of detecting (and validating) the presence or absence of adventitious levels of plant residue in faecal material. Also, a nested PCR or an equivalent system could be used to enhance the PCR signal.

The use of faecal DNA to determine components of the diet of kangaroos

Plant residue in faecal material can be detected by PCR amplification but the choice of primer pairs remains critical. The use of faecal DNA is still only in its developmental stage in terms of determining the composition of the diet of kangaroos. The technology has previously been used to determine components in the diets of sea lions (Deagle *et al*., 2005) and brown bears (Murphy *et al*., 2003). The diets of animals (and kangaroos) in which a variety of food is available are likely to be complex. Where a herbivore grazes a cultivated pasture, usually containing only a limited number of pasture species, the diet is relatively simple. The more the sources of DNA in any sample the greater the difficulty in isolating individual sources of DNA (Jarman *et al*., 2002).

In this study, seven samples were PCR negative and five samples each contained an amplified 'plant' band that could not be confidently identified. When DNA is of poor and low quantity, it can produce erroneous results (Morin *et al*., 2001; Nsubuga *et al*., 2004). Compared with plant material, the conditions for PCR amplification on faecal DNA are more technically demanding given the propensity for non-specific amplification. Spiking faecal DNA with plant reference DNA (data not presented) largely eliminated non-specificity, which substantiates the conclusion that low levels of target sequences is a contributing factor to diminished PCR response.

Diet selection based on faecal DNA analysis

Although kangaroos preferentially graze grasses (Taylor, 1983; Short, 1986; Dawson and Ellis, 1996), they also consume a variety of shrubs and subshrubs (Short, 1986; Wann and Bell, 1997), as was shown in this study. Of particular importance is that the plants selected for this study were those considered palatable to sheep, indicating

that there would be direct competition between sheep and kangaroos for these particular forage species. Determining the extent of competition between sheep and kangaroos will require increasing the number of plant species DNA fingerprinted. Genetic analysis of faecal matter could potentially provide a quantitative estimate of diet composition rather than just qualitative information on the presence or absence from the diet if the amount of (faecal) DNA from each animal is proportional to the mass (of the particular plant) in the diet. Recent testing did provide a rough estimate of the proportion of the fish present in the meals fed to sea lions (Deagle *et al*., 2005).

Despite the limitations of the technology, DNA fingerprinting has been a useful tool to assess diet selection by kangaroos in a rangelands environment. A logical progression would be the development of a system of quantification of plant species in the faecal matrix.

Acknowledgements

This study was made possible by funding provided by the Rural Industries Research and Development Corporation (Australia), ChemCentre and Curtin University of Technology. We thank Mr Michael Walker and Mrs Katarzyna van der Reest for their assistance in the DNA analytical work. Mrs Beverley Pratt is thanked for her assistance in the collection of plant and faecal material.

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