Diets of the Macropodidae inferred through Dental Microwear Texture Analysis

by

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Thesis
Submitted to Flinders University
for the degree of

Doctor of Philosophy

College of Science and Engineering

December 2018

Dedicated to Heather Perchard (1926–2015).

Contents

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed ……

Date: 15/9/2017

Abstract

Kangaroos are the principal endemic vertebrate herbivores of Australia. Evolving in response to increasing aridity in the late Neogene, Pleistocene forms are dominated by two main groups; long-faced macropodines, and short-faced sthenurines. Differences in functional morphology suggest that diet differentiates these groups. Macropodine kangaroos possess high-crowned molars, inferred as an adaptative response to the wear produced by consuming abrasive grasses. In contrast the complex morphology of sthenurine molars is interpreted as an adaptation to browsing on tougher leaves of shrubs and other dicotyledonous plants. Skull length, which most obviously distinguish these groups is also considered a dietary adaptation. The longer diastema between the molars and incisors of macropodines assist in orally manipulating long grasses, while the shorter sthenurine skulls increase the forces of mastication required to break down tough browse.

Inferring diet through functional morphology is, however, limited. Morphology often may only reflect the most restrictive element of an animal's diet. Moreover, it tells us little about finer-scale differences in diet within or between species. Such fine-scale differences are key to understanding more about these species. Greater understanding of diet can inform about niche partitioning, where different food resources are utilised by different species, facilitating greater regional diversity than competition would usually allow. Understanding dietary change over time can inform on how adaptable species are to changes in vegetation, and potentially feed into conservation of living kangaroos. A narrow diet may leave a species vulnerable to extinction, either directly through loss of that resource, or indirectly through flow-on effects of being bound to the physiological or geographical constraints of that resource. This has particular implications for sthenurine kangaroos, all of which went extinct in the late Pleistocene. Their extinction, alongside numerous other "megafaunal" groups, is the subject of ongoing debate in relation to the influence of climate change and human

interaction. Understanding diets of sthenurines, as well as those of all kangaroos, may provide key information on these extinctions, and by understanding how plant resources have been used by different species in the past may help manage kangaroo populations today.

Here we investigate the diets of kangaroos through Dental Microwear Texture Analysis. This method operates by considering the impacts that food make on tooth enamel as animals chew their food. Physical characteristics of different foods, alongside grit adhering to, and phytoliths within foods, alter markings produced on tooth enamel during occlusion. To analyse these markings, high-resolution 3D scans are taken of the molar wear facets. Algorithms are applied to scans to quantify differences in surficial characteristics, and have been shown in numerous mammalian groups to distinguish between species with different diets.

To embark on a study of macropodid microwear first requires that a baseline is established, to allow the diets of extinct kangaroos to be inferred by comparison with living kangaroos of known diets. Such baselines are necessary for each group being studied to ensure that broad cranio-dental differences between mammalian groups do not bias results. To construct this baseline, and get a better understanding of modern kangaroo diets, we collated all published literature of dietary intake for kangaroos. A coarse classification was then established to sort living species into dietary groups: fungivores, browsers, mixed-feeders and grazers. Most species were classified as mixed feeders, which contrasts to similar analyses of herbivorous groups elsewhere. This is a possible adaptation to the often unpredictable environmental conditions in Australia. Very few species are specialist browsers, which supports the notion that this niche may have been largely filled by the now-extinct sthenurine species.

As microwear data collection began, a hurdle was encountered regarding comparability of data collected on different instruments. To minimise the effects of this, a series of filters were

established to allow comparability between instruments. This led to further consideration of variation within species. Such questions have been considered elsewhere, with the result being that modern microwear sampling is limited to avoid effects of intraspecific variability. Such sampling practices, however, are limiting for palaeontological purposes, where sample sizes are inherently low. In addition, some differences within species, such as geographic origin of modern specimens, may reflect dietary differences that could assist in fine-tuning the dietary signal. With these matters in mind, we turned to general linear mixed-modelling to incorporate intraspecific factors into models of differences between species. This method includes factors in models only when they can improve the ability of the model to describe the data. Models generated indicated a small number of factors, including facet scanned and ecoregion of specimen origin to have the greatest effect on microwear data. Most important, however, appeared to be the inclusion of each specimen modelled as a random effect, likely to encapsulate natural inter-individual variation. Utilising modelling thus enables broader sampling practices at the same time as incorporating intraspecific variability where present, to enhance our ability to differentiate between species with different diets.

Turning finally to dietary analysis of macropodid microwear, analyses of modern species suggested that only 10 of 28 dental microwear texture analysis algorithms utilised showed any ability to differentiate between species with known differences in diet. Palaeontological analyses added the diverse assemblage from Victoria Fossil Cave, Naracoorte, South Australia. Findings revealed that most sthenurine species were indeed browsers, but that differences were evident between species. Some sthenurine species were strict browsers, while others had more mixed diets or may have been frugivorous. Considerable dietary overlap was also present between most sthenurine and macropodine kangaroos from the same deposit, supporting dietary flexibility as a core feature of all kangaroo diets. These results

suggest that sthenurine extinctions were unlikely the result of any climate driven vegetation change.

Acknowledgements

First and foremost is Gav, who has been a great mate throughout my tenure. Gav's ability to reign in the more ridiculous suggestions, and ask the relevant questions kept this project rolling. Also his understanding about broader palaeontology methods, music, and what's wrong with the AFL and the Cricket Australia provided essential distractions. His inability to plan ahead for gigs also was also handy. Pete Ungar was also essential to the project, as well as the anthros at Uni Arkansas, and other troops across the US. Thanks to the field crews who allowed me along on their jaunts over there; The Gray Fossil Site, The Los Angeles County Museum of Natural History (both dino and mammal crews), and the Denver Museum of Nature and Science. Cheers to my US mates Dom, Keith, Eric, Jess, and the girl who hit my car and inadvertedly paid my way across the country. Also thanks to my little bro for helping me out a couple of times when I was broke, and my grandpa, whose inheritance paid for most of my trip.

To the extended Flinders palaeo lab, a better bunch of jerks is hard to find. Our lab has grown continuously during my tenure, and the diverse sets of ideas and field trips that crop up definitely keep the place interesting. Special thanks to Aaron, Aidan, Amy, Carey, Diagonally, Jake, Kailah, Lisa and Matt, for their commitment to having a beer whenever it was required. Special thanks to Ben King because he asked me to. Thanks to Rach, Elen and Alice for sharing my pain. Thanks to Trev for putting your hand up as a co-supervisor, despite being inexperienced in kangaroos or dental microwear. Thanks to Gully for helping keep the various microwear bits and bobs in order. Thanks to Michael *et al.* at OneByte for always saving me the last cheese and spinach roll.

Beyond the lab thanks to everyone who helped keep me sane over this period. In no particular order all these people were essential: Shano, Barman, Randa, The Warradale Cricket Club,

Flinders University Ultimate, The Hot Beachezz, Three D Radio, and my chooks. To all my real friends you know who the fuck you are.

Thanks to Jemma and Trev for reading a draft of this. Technical acknowledgements can be found in the relevant chapters.

Preface

Chapters 2–5 of this thesis are prepared as stand-alone papers for scientific journals. As such there is some overlap between chapters, and minor formatting differences as was required by some journals. Chapters two and three have previously been published but have been modified from those forms in response to assessor criticisms.

List of published papers and manuscripts

Chapter 2. Arman, S. D. and G. J. Prideaux (2015). Dietary classification of extant kangaroos and their relatives (Marsupialia: Macropodoidea). *Austral Ecology* **40,** 909–922.

Chapter 3. Arman, S. D., Ungar, P. S., Brown, C. A., DeSantis, L. R., Schmidt, C., and G. J. Prideaux (2016). Minimizing inter-microscope variability in dental microwear texture analysis. *Surface Topography: Metrology and Properties* **4,** 024007.

Chapter 4. Arman, S. D, Couzens, A, Ungar, P. S., and G. J. Prideaux (in preparation) Incorporating intraspecific variability into Dental Microwear Texture Analysis.

Chapter 5. Arman, S. D., and G. J. Prideaux (in preparation) Dietary diversity in Pleistocene Kangaroos of south-eastern Australia.

Chapter 1

Dental Microwear

'[*S*]*ince Laetoli and Hadar have very close tooth wear and that microscopic examination of the teeth from Olduvai has showed few striations we know that these hominids did not unfortunately drop their food in the sand.'*

-Pierre-François Puech *et al.* 1983*

1.0. Context

This chapter provides an overview of the theory underpinning the data chapters of this thesis. It covers the origins of dental microwear, theories regarding feature formation and the ongoing evolution of methodologies.

*Puech, P.-F., H. Albertini and C. Serratrice (1983). Tooth microwear and dietary patterns in early hominids from Laetoli, Hadar and Olduvai. *Journal of Human Evolution* **12,** 721–729.

Dental Microwear

1.1. Origins of microwear, historical and functional

The origins of dental microwear studies are found in the work of early palaeontologists and anatomists in their attempts to understand the complexities of mastication. In 1878, John Ryder published an exemplary monograph on comparative anatomy, ontogeny and function of mammalian dental systems. Amongst his observations, he discussed the motions of the jaws of different mammals, in part based on striations seen on teeth, which he argued implied the directions of motion in chewing (Ryder 1878). From that point onwards many studies attempting to elucidate jaw movements in mammals included discussions of wear on teeth (e.g., Butler 1952, Mills 1978, Calandra *et al.* 2016a). Initially these markings were considered as evidence of tooth movement, rather than of dietary inference, however microwear research expanded when morphometric measurements and observations of wear were tied to dietary characteristics (e.g., Kay 1975, Rosenberger and Kinzey 1976, Seligsohn and Szalay 1978).

A number of fundamental protocols for later research on dental microwear were also established through studies on the movement of teeth in occlusion; the process of jaws closing and teeth coming together. The occlusal movement of jaws were more formally designated by breaking them down into ‘phases’ that describe their motion (Hiiemae 1978). As the terminology developed, phases were applied in particular reference to the interactions between teeth, with phase I describing where teeth come together and food is cut on sharpened lophs, in contrast to phase II where food is crushed on planar faces of teeth (figure 1.1, Hiiemae 1978, Mills 1978, Kay 1981). Importantly, phase II involves movements of teeth in lateral, medial and anterior movements as food is broken down, and microwear created (Kay 1981). Subdividing teeth into areas involved in phases I and II also led to an

understanding of wear facets on teeth. Facets are individual regions on teeth considered to abut areas on opposing teeth during occlusion, so facet 1 on an upper molar occludes with facet 1 on a lower molar (Butler 1952). The importance of this to microwear research is in establishing where to sample microwear, particularly given the numerous schemes that existed prior to the work of Hiiemae (1978). Focusing firstly on phase II facets, and then on a small number of these, allowed greater comparability between studies. This is not to say that sampling has been consistent since that point, but at least a scheme for documenting sampling was established.

Figure 1.1: Phases in occlusion: Phase I (left) where food is cut on crests, and Phase II (right) where food is crushed on lophs. Modified without permission from Thewissen *et al.* (2011).

From the late 1950s onwards, work also began on one of the most central arguments within microwear analysis: what actually causes microwear formation? One early explanation was that wear was caused by phytoliths, hydrated silica particles which abound in a cells of some plants, particularly grasses. This idea was supported by the observation of fractured phytoliths in sheep faeces, as well as hardness tests of phytoliths, enamel and dentine (Baker *et al.* 1959). These hardness tests were considered crucial as they allow the harder phytoliths to leave impressions in the softer enamel, though conflicting findings have been made more recently (Sanson *et al.* 2007, Lucas *et al.* 2013). The phytolith theory still has some traction

however, with support coming in experimental work, which even suggested that the shape of some microwear features could be directly attributed to phytoliths of individual plants (Gügel *et al.* 2001, Merceron *et al.* 2016). Moreover, phytoliths may actively remove enamel rather than simply deforming it (Xia *et al.* 2015).

Sand and dust consumed with food has also been implicated in microwear formation, again on grounds of their greater hardness than enamel (Lucas *et al.* 2013), as well as being present on many foods. Support for the role of sand in microwear formation has come from experimental feeding (Covert and Kay 1981, Peters 1982), studies on moles that consume sand-encrusted earthworms (Silcox and Teaford 2002), comparisons of the size and shape of sand grains to microwear features (Ryan 1979b, Ryan 1979c), and studies on the physical and mechanical properties of enamel, sand, and phytoliths (Maas 1991, Maas 1994, Lucas *et al.* 2013). In addition to sand, airborne dust on foliage also appears a significant factor, and the amount of dust present on leaves is affected by the height of leaves as well as environmental factors (Teaford *et al.* 1994, Ungar *et al.* 1995, Nystrom *et al.* 2004).

Rather than substances consumed, other workers have suggested that tooth-tooth contact, particularly during thegotic tooth-sharpening, may be the source of microwear (Ryan 1979a). However, this idea is undermined by the prescence of clear dietary signals (Teaford and Walker 1984) and microwear on non-occlusal surfaces (Ungar and Teaford 1996). Others have suggested that microwear may indicate how teeth may be used for ‘auxillary activities’, such as preparing hides, particularly for hominids (Brace *et al.* 1981, Krueger *et al.* 2008). This, however, overlooks the existence of microwear in numerous taxa less likely to be involved in similar behaviours, such as bovids (Scott 2012), kangaroos (Prideaux *et al.* 2009) and even fish (Purnell and Darras 2015).

Turning finally to food itself, interpreting microwear formation is plagued by a single self-evident fact. Breaking down foods in chewing is enabled by teeth being harder than the foods they chew. If foods are softer than enamel, then how are they able to leave microwear on teeth? The answer to this question lies in part in the chewing cycle itself, where animals, particularly herbivores, will undertake thousands of chews per day (Xia *et al.* 2015), and so repeated passage of foods across surfaces incrementally adds microwear features. "Even the Grand Canyon was produced by water" (P. Ungar, pers. comm., July 2015). Indeed, a number of soft food items have been shown in experimental conditions to produce microwear (Hua *et al.* 2015, Xia *et al.* 2015, Daegling *et al.* 2016). Foods are also rarely homogenous, so it may be that microwear records harder parts of foods eaten in order to access more palatable softer foods, such as twigs eaten while consuming leaves. This does, however, create the possibility that microwear analyses will be biased towards the hardest parts of an animal's diet (e.g., Puech 1979, Solounias and Semprebon 2002, Scott *et al.* 2006). In addition, some species are known to 'pre-process', or physically modify foods so the final object interacting with molars may be quite different from those found in the environment. For example, stem stripping in *Gorilla* (Ryan 1981), using stones to break open nuts in *Cebus* (capuchin monkeys) (Fragaszy *et al.* 2004), or indeed cracking of nuts in potoroine kangaroos (Prideaux 1990), all of which could result in different microwear signatures being present than if the same foods were eaten without pre-processing.

The processes discussed above are not necessarily opposed, however, and it is possible or even likely that most play at least some part in microwear formation. Regardless of aetiology, there is undoubtedly some sort of signature present in microwear that aligns with diet across a range of groups (Calandra and Merceron 2016). The level at which diet can be differentiated varies by methodology, available samples, and taxonomic group, amongst other issues, but some general statements regarding dietary differentiation can still be maintained.

Herbivorous species are typically seen in a browsing–grazing continuum, with browsers (dicot leaf specialists) and grazers (grass specialists) being at either end, and mixed feeders which consume both grass and leaves, in the centre (Gagnon and Chew 2000). The consumption of harder food items, such as twigs and stems by browsers, is thought to typically result in surfaces with more 'pits', while the higher proportion of sand on and phytoliths within grasses produce more 'scratches' on grazer teeth (e.g., Ungar 1996, El-Zaatari *et al.* 2005, Ungar *et al.* 2012). In addition, feeding on hard objects, such as nuts and seeds (e.g., Teaford and Runestad 1992, Galbany *et al.* 2005b, Scott *et al.* 2005), insects (Teaford and Robinson 1987, Teaford and Runestad 1992), or tubers and roots with the associated sand ingested (Daegling and Grine 1999, Ungar *et al.* 2006), have been shown to result in distinctive microwear signatures. Dental microwear of carnivores relates to how much bone is consumed, with hard bone creating more surface pitting than softer flesh (e.g., Robson and Young 1990, Schubert *et al.* 2010, DeSantis and Haupt 2014).

1.2. Methodological Development

The development of microwear methodology can be broadly divided into the three main sources of data collection used: Light Microscopy, Scanning Electron Microscopy, and Dental Microwear Texture Analysis, which analyses data from scanned surfaces.

1.2.1. Light Microscopy

Although details are scant, it seems most likely that the observations of Ryder (1878) were made with the assistance of a standard light microscope. Light Microscopy (LM) may be considered the simplest methodology used because the surfaces themselves are observed, not directly recorded, although there are exceptions (e.g., Walker *et al.* 1978, Puech *et al.* 1983, Morel *et al.* 1991), with generally poor results. Without a recorded surface, LM studies cannot take quantified measurements, resulting in the microwear signature being vague, based on descriptions (Puech *et al.* 1983), presence/absence of microwear (Ungar and

Teaford 1996), or broad categories (Walker *et al.* 1978). The question of subjectivity of LM microwear analyses has been addressed by some authors. Analyses of variability in LM microwear data collected from a large taxonomic range showed no significant difference between two observers in pit and scratch counts, despite demonstrable differences in raw data collected (mean scratch error 9.9%, mean pit error 11.0%) (Semprebon *et al.* 2004). Others have demonstrated 36% intra-observer error between reiterated testing, mostly found in inexperienced observers and likely due to learning (Mihlbachler *et al.* 2012). When considering differences between observers, significant differences were evident in every case, and neither experience nor dietary group studied were considered to be causal factors (Mihlbachler *et al.* 2012). Having a single researcher take all measurements may limit error, and instead of using published data, researchers were recommended to collect baseline samples themselves, assisted through large shared image banks (Mihlbachler *et al.* 2012). Other issues of LM microwear are related to the limited depth of field and resolving power of light microscopes (Ungar *et al.* 2008b). This is not to say that the method has lost its appeal, as is evident with recent work being undertaken on equids (Solounias and Semprebon 2002), squirrels (Nelson *et al.* 2005), and carnivorans (Bastl *et al.* 2012). Modern LM microwear adherents argue that although the results are more subjective, the lowered time and cost constraints provided by light microscopy enables a larger sample to be collected, providing value for the technique over other methodologies where the time and expense of scanning generally results in lower sample sizes being acquired (Solounias and Semprebon 2002).

1.2.2. Scanning Electron Microscopy

Microwear studies using Scanning Electron Microscopy (SEM) are the most common in the literature, and have focused on a large range of taxa, including bats (Silcox and Teaford 2002), carnivorans (Anyonge 1996, Pinto-Llona 2013), marsupials (Robson and Young 1990), reptiles (Maas 1991), rodents (e.g., Rensberger 1978, Puech *et al.* 1986, Silcox and

Teaford 2002), ungulates (e.g., Maas 1991, Solounias *et al.* 1994, Mainland 1998), and particularly primates (e.g., Gordon 1982, Teaford and Robinson 1989, Estebaranz *et al.* 2009), hominids (e.g., Brace *et al.* 1981, Grine 1984, Ungar *et al.* 2006) and humans (e.g., Teaford and Tylenda 1991, Ungar and Spencer 1999, Pérez-Pérez *et al.* 2003). The use of SEM microwear in dietary analysis can be traced to Walker *et al.* (1978) and Rensberger (1978), with the latter probably taking precedence being based on a 1974 conference. A major advantage in using SEM is that the images themselves can be measured, published and shared, leading to greater transparency and repeatability (Gordon 1982).

The potential of greater objectivity through shared SEM images and data is undermined by variations in scanning and quantification parameters between studies. Scanning variability can be demonstrated in the magnification of the scan, which varies from 35x (e.g., Ryan 1979b, Ryan 1981, Walker 1981) up to 6000x (Peters 1982) but more typically between 100x and 500x (e.g., Grine and Kay 1988, Anyonge 1996, El-Zaatari *et al.* 2005). Another issue of concern is angle of illumination, which is considered to play a major role in feature identification when regarding whether light falls on a surface perpendicular or parallel to microwear features (e.g., Grine 1986, Ungar and Grine 1991, Scott *et al.* 2005). This variability was even used as a quantification method, by considering the intensity of light at five degree intervals across SEM images (Grine and Kay 1988). Meanwhile, advances in technology have vastly improved the clarity and depth of field of SEM images (King *et al.* 1999b), which, while improving the ability of researchers to discern features, also limits comparability of results between studies. Finally, features of the microscopes themselves such as the voltage and type of electrons used (backscattered versus secondary), or thickness and properties of coating materials on specimens, may influence results (Ungar *et al.* 2008a).

The manner by which features are quantified by SEM microwear workers also varies greatly, from purely subjective comparisons (Covert and Kay 1981), to manually measuring features

with calipers (e.g., Peters 1982, Ryan and Johanson 1989, Ungar and Grine 1991), digitising tablets (e.g., Teaford and Robinson 1989, Maas 1991, Rafferty *et al.* 2002), and more recently a range of automated and semi-automated software (e.g., Ungar 1995, Lalueza *et al.* 1996, Pérez-Pérez *et al.* 2003). When measuring features in 2D space, SEM methods also assume surfaces to be planar and parallel to the recorder, which is hard to maintain in complex surfaces such as teeth, particularly as surfaces horizontal to the electron beam show poor contrast (Gordon 1982). This was, however, partially overcome through the use of a digitising program that corrected for foreshortening of features (King *et al.* 1999a).

The SEM microwear literature contains a number of key measures for distinguishing diets. Ungar *et al.* (2006) indicated that the four most used measures in SEM-based studies were pit percentage, pit width, scratch width, and mean-orientation vector length (feature orientation and length). Classification of scratches though is varied. They have been defined as having a length greater than its width (Gordon 1982), or having a length to width ratio of 3:1 (Galbany *et al.* 2005a, Galbany *et al.* 2005b), 4:1 (e.g., Grine 1986, Teaford and Runestad 1992, Galbany *et al.* 2009), 10:1 (Teaford and Walker 1984, Teaford 1985), or recognised entirely subjectively (Bullington 1988). An alternative to the pit/scratch dichotomy was also used by Ungar and Spencer (1999), who considered all features identically, without distinguishing between pits and scratches. Indeed, the varieties of pit and scratch categorisation is due in some part to their inherently arbitrary distinction, and that pits and scratches are, 'not categorically distinct manifestations of different activities, but rather are opposite poles on a microwear continuum' (Gordon 1984; p1044).

In addition to pits and scratches, a range of other terms have also been included, such as crenulations (Puech *et al.* 1986), depressions and microflakes (Ryan and Johanson 1989), gouges (Mainland 1998), fissures, flaked pits, furrows, pebbly texture and polish (Rensberger 1978). Some authors also chose to distinguish between small and large features, such as Ryan

(1981) who considered features of < 0.7 mm diameter to be small, while Teaford and Runestad (1992) and Solounias *et al.* (1988) chose 4 and 5 μm respectively, as their cut-off points, based upon the average enamel prism size for the groups being studied. Others have used a ratio-based approach (Mainland 1998), or separated pits on size, and scratches on ratios (Nystrom *et al.* 2004). These efforts were presumably undertaken to try to encapsulate further detail of surfaces, but in reality, introducing new variables can often confound the situation and make comparisons between studies harder (see figure 1.2).

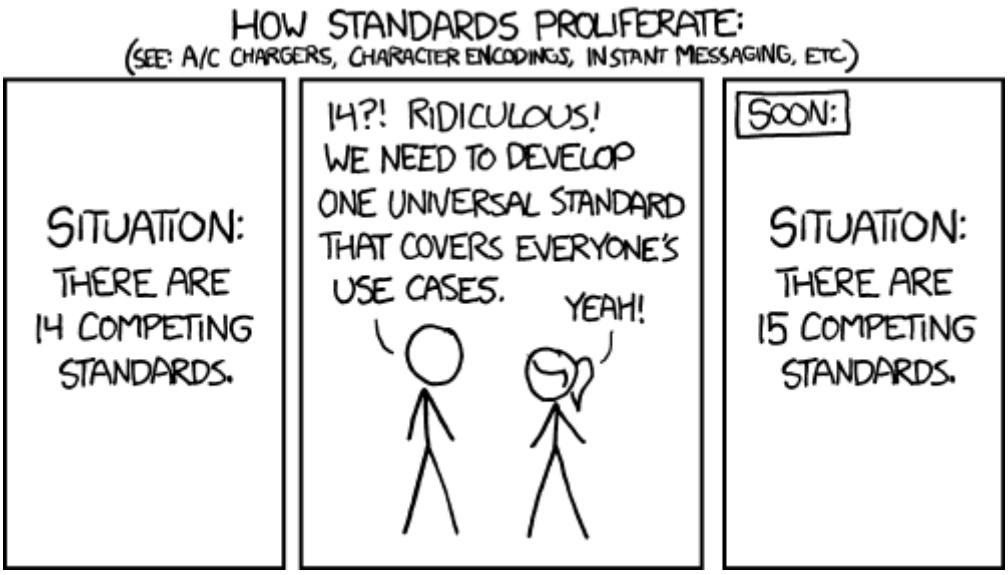

Figure 1.2: How microwear variables proliferate. Source: 'Standards' http://xkcd.com/927/

Concerns over subjectivity of quantification led a number of authors to consider differences between operators, or even between scans taken by a single operator. Repeated quantification of a single image over time by a single operator resulted in an average of 7% difference (Grine *et al.* 2002), while inter-observer error showed a mean discrepancy of 9% between operators, and high variance in this across different parameters (Grine *et al.* 2002). A mean difference of 19% between methodologies was also observed, but this was likely due to inherent differences (e.g., scale) between techniques (Grine *et al.* 2002). Intra-observer error on buccal surfaces of teeth also found error averaged 5% and was influenced by observer

experience (Galbany *et al.* 2005a). A comparison of the results of the two microwear-measuring researchers revealed no significant difference (Estebaranz *et al.* 2009), but given that each was gathering data on distinct samples this somewhat limits the veracity of the comparison.

1.2.3. Dental Microwear Texture Analysis

Dental Microwear Texture Analysis (DMTA) seeks to overcome inter-observer error by utilising algorithmic quantification of surfaces, rather than subjective quantification or manual counts of features. Rather than images, this requires either 2D profiles (see Kaiser and Brinkmann 2006), or 3D scans (see Ungar *et al.* 2003) to be taken of the surface in question. Overwhelmingly, most researchers are now turning to confocal microscopy to acquire 3D scans (e.g., Ungar *et al.* 2003, Scott *et al.* 2005, Calandra and Merceron 2016). Confocal microscopy works by focusing laser light to the plane of a microscope objective (Ungar *et al.* 2003). Light is reflected back to a photodiode only from the plane of focus, which creates a series of points for that plane, which when combined across focal lengths creates a three-dimensional point cloud representing that surface (Ungar *et al.* 2003). More recently, white light scanning confocal microscopes have also been used, which have greater axial resolution than laser light (Ungar *et al.* 2007).

The scans produced require editing before data can be collected from them. This entails the removal of non-microwear features, such as dust, as well as ‘spikes’, which are aberrations generated by the surface detection and modelling process (Calandra and Merceron 2016). The scan is then analysed through either Scale-Sensitive Fractal Analysis (SSFA), or Surface Texture Analysis (STA). It should also be noted that in both SSFA and STA, non-normal distributions of data necessitate non-parametric comparative statistics be utilised (e.g., Scott *et al.* 2005, Scott *et al.* 2006, Henry *et al.* 2012). Others use data trimming to remove outliers,

or use rank (e.g., Krueger *et al.* 2008, Ungar *et al.* 2008a, El-Zaatari 2010) or logarithmic (Schulz *et al.* 2010) transformations before parametric analysis (Calandra *et al.* 2012).

Scale Sensitive Fractal Analysis

SSFA methods are largely based on considering how a variable changes when the scale of measurement is altered (Ungar *et al.* 2003). One benefit of this method is that it is measurement-independent, so as long as they are roughly comparable, data collected on different profilers can be compared (though see Chapter 3 herein). Indeed, SSFA measures the difference in a variable between scales of measurement, and in doing so demonstrates why methods that measure at a single scale tell only part of the story, and are problematic to compare between different profilers. There are five SSFA algorithms commonly used in DMTA (see Scott *et al.* 2006 for a more detailed explanation).

Area-Scale Fractal Complexity

Area scale fractal complexity (*Asfc*, or 'complexity') is at the core of SSFA. It is measured by considering the relative area of a surface across scales of measurement (Scott *et al.* 2005). Surfaces with numerous features, such as extensive 'pitting', have greater changes between scales than simpler, flatter surfaces (Ungar *et al.* 2003). Typically, this separates high-complexity browsers from low-complexity grazers (Scott 2012). Complexity is particularly useful in identifying diets based on hard and brittle foods, which have higher and more variable complexity values than animals with tough food diets (Scott *et al.* 2006). This variable also provides greater resolution within traditional dietary categories. Herbivores that include fruits, tubers etc., in their diet have greater complexity values than those which specialise on leaves alone, while grazers have the lowest complexity values of all (Ungar *et al.* 2007).

Scale of Maximum Complexity

Scale of maximum complexity (*Smc*) is related to complexity by considering the scale at which the greatest change in relative area (i.e. the greatest complexity) is found (Scott *et al.* 2006). The scale of maximum complexity indicates the size of most microwear features (Scott *et al.* 2009). This measure, though, is limited, because even in large datasets, variation is minimal, largely uniform, and does not follow dietary lines (R. Scott pers. comm., November 2014).

Heterogeneity of Area-Scale Fractal Complexity

Heterogeneity of *Asfc* (*Hasfc* or 'heterogeneity') considers how complexity varies across surfaces, by dividing the initial scanned surface into smaller and smaller subregions and calculating the complexity for each of these (Scott *et al.* 2006). The difference between each subregion and the median is calculated and the median of these for all cells is then taken as the value for heterogeneity. Heterogeneity in complexity has been previously shown to distinguish between diets that are coarsely similar, but helping distinguish species with a more heterogeneous or mixed diet (Scott *et al.* 2006). For example, the occasional consumption of hard food items may result in a more heterogeneous microwear pattern without effecting the overall complexity of the tooth.

Length-scale Anisotropy of Relief

Length-scale anisotropy of relief (*epLsar* or 'anisotropy') compares the relative length of cross sections across 5˚ intervals over the scan (Ungar *et al.* 2003). These are then normalised by the 'exact-proportion' method where each length is divided by the sum of all lengths to remove the effects of deep microwear features (El-Zaatari 2010). Heavily scratched teeth will have higher anisotropy due to increased length of profiles when cross sections intersect the many 'valleys' of roughly parallel scratches in a scan (Scott *et al.* 2006). Anisotropy is used

by most researchers, and often inversely correlates with complexity (Ungar *et al.* 2007). This relationship can be best explained by reference to the pit and scratch dichotomy used in SEM analysis. Surfaces with many pits, such as those of browsing herbivores, have highly complex surfaces with little anisotropy (high *Asfc* and low *epLsar*), while surfaces with many parallel scratches, such as those of grazers have less complex, highly anisotropic surfaces (low *Asfc* and high *epLsar*) (Ungar *et al.* 2007).

Textural Fill Volume

Textural fill volume (*Tfv*) is calculated by the volume that is required to fill a scanned surface (Scott *et al.* 2006). Because this is likely to vary due to overall concavity of structures, the algorithm considers the difference between the volume to fill the surface with a cubes of a set size (10 μm), and the total volume to fill that surface, enabling the texture to be quantified independent of the overall shape of the surface (Scott *et al.* 2006). In addition, Scott *et al.* (2009) considered this with finer (2 μm) cubes under the name of fine textural fill volume (*Ftfv*), to reveal differences in the scale of features (Scott *et al.* 2009). Textural fill volume is thought to be better able to distinguish between foods that are eaten in a similar way, but have different fracture properties (Scott *et al.* 2009).

Surface Texture Analysis

Application of STA to dental microwear analysis has been commenced after the establishment of SSFA, but it is essentially a simpler technique. It was developed from existing International Organisation for Standardization (ISO) algorithms used in engineering to describe surficial properties of materials (Calandra *et al.* 2012). Practitioners of STA methods favour them because of the multiple available algorithms, which enable greater investigation of specific questions regarding the aetiology of microwear (Calandra *et al.* 2016a). There are multiple algorithms available, which can be tailored to specific questions,

such as differentiating hard diets, including seeds, bark and insect cuticle, from wear caused by small particles like phytoliths and dust (Calandra *et al.* 2012). However, STA may be less informative regarding actual dietary differentiation than SSFA, because of the scale over which some measures operate (Calandra *et al.* 2012).

After initial explorations in understanding microwear through ISO by Kaiser and Brinkmann (2006), the definitive STA analysis of Schulz *et al.* (2010) used 30 distinct algorithms, though others have focused on less than 30, usually for technical reasons (e.g., Kaiser and Brinkmann 2006, Purnell and Darras 2015, Calandra *et al.* 2016a). All of the ISO algorithms utilised in STA can be considered in the six categories below, following Schulz *et al.* (2010) and Purnell *et al.* (2013b). It should be noted that these two papers differ somewhat in which of the parameters were used, as well as from the ISO list (which now numbers well over 30), in terms of their classifications within categories (ISO 2010). Nonetheless, as Schulz *et al.* (2010) and Purnell *et al.* (2013a) provide classifications best suited for DMTA, they will be used here.

Height

Seven height-based variables are used in STA, and are uniform between authors and the ISO classifications (ISO 2010, Purnell *et al.* 2013a, Calandra *et al.* 2016a). Some of these are ultimate measures such as maximum height (*Sz*), or maximum peak height (*Sp*), or describe the distribution of height across a surface, such as average height (*Sa*), or skewness of height (*Ssk*) (Purnell *et al.* 2013b). Height parameters are considered to be descriptive of the basic properties of surfaces (Calandra *et al.* 2016a), as well as indicating consumption of high proportions of large hard particles (Calandra *et al.* 2012).

Volume

Volume based variables measure the 3D area of either the surface or its inverse, such as material volume (*Vm*), which is simply the volume of the surface, volume of the peaks (*Vmp*) or of the void in the valleys (*Vvm*) (Purnell *et al.* 2013b). Peaks and valleys themselves are considered in reference to 'core material', calculated using the Areal Material Ratio Curve, which describes the cumulative proportion of the surface for each given height (Purnell *et al.* 2013a). Volume parameters, like height, are considered to be indicative of the general shape of the surface, and all four volume variables used by Purnell *et al.* (2013b) have been found to differentiate between dietary groups. Material volume is also thought to be inversely correlated with the SSFA variable textural fill volume (Calandra *et al.* 2012).

Feature

Feature-based variables are commonly used in STA, but vary considerably in how they are utilised by different researchers. Peak density (*Sha*) refers to the number of peaks, while the five point height (*S5z*), and depth (*S5v*), average the five highest and deepest points, respectively (Calandra *et al.* 2012). These provide an average for the largest features on the scan, with consumption of large, hard particles, such as seeds and bark, likely to result in higher five-point depth values (Calandra *et al.* 2012). Closed dale area (*Sda*) and Closed hill area (*Sha*) are thought to indicate feeding on small hard objects, while peak density is highest where enamel is being chipped by large hard items, and lowest when smaller particles are ingested (Calandra *et al.* 2012).

Material Ratio

The five material ratio variables all relate to a specific measure in relation to the core material of the surface (see above). Examples of material ratio variables include mean height (*Spk*) or proportion (Smr1) of the peaks above the core material (Purnell *et al.* 2013a). To date, these

have only been utilised by Calandra *et al.* (2016b), who found four of the five variables considered to correlate with dietary groups.

Spatial

Spatial measures of surfaces are designed to correlate repeating patterns across a surface, and three of these variables have been applied in STA (Schulz *et al.* 2010). Of these, only texture direction (*Sal*), which calculates the main direction of textures, has shown any utility, where it was considered to reflect the direction of motion of food in occlusion (Calandra *et al.* 2016a).

Hybrid

Two hybrid parameters have been used in STA, root mean square gradient of the surface (*Sdq*), and developed interfacial area ratio (*Sdr*). Despite being included in a number of analyses, these parameters have not demonstrated dietary distintion.

1.3. Conclusion

Over a century has passed since the first observations of wear striations on teeth (Ryder 1878), and nearly 50 years since the establishment of dental microwear as a dietary proxy (Rensberger 1978). In that time we have seen dental microwear methods used across a range of (mainly) mammalian groups to infer their diets. More importantly though we have seen continual development and fine-tuning of methodologies. Researchers have not been content to ignore difficult questions, such as subjectivity of methods and feature formation. Considering these questions has helped develop methodology and drive change, even when it meant abandoning older paradigms.

However, methodological development of microwear analysis does not stop at DMTA. There are still questions that need addressing and continual testing to determine which variables are most effective at discriminating diet, which other factors may effect microwear signals, and

at what scale diets can be differentiated. At the same time workers are continuing to develop the modern baselines necessary to undertake palaeontological inference. Microwear also does not stand alone in palaeodietary inference and is being increasingly united with other palaeodietary proxies, such as morphology and stable-isotope analysis.

The future of dental microwear will, like all methodologies, depend on its utilisation. If it can continue to refine our understanding of the diets of extinct mammals and the world around them, it will likely remain in use for the foreseeable future. Refinement though, requires further consideration of how data is collected, analysed and interpreted. To remain a viable dietary proxy the microwear signal must override any methodological noise, and provide a useful and meaningful insight into one of the most fundamental elements of an animal's life, its diet.

1.4. Acknowledgements

Thanks to Grant Gully for useful criticisms of the draft of this chapter.

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Chapter 2

Dietary classification of extant kangaroos and their relatives (Marsupialia: Macropodoidea).

'*They are not carnivorous, and subsist altogether on particular flowers and grass.'*

-Watkin Tench, 1793*

2.0 Context

This chapter classifies the diets of living kangaroos. Dietary classification provides a broad framework for understanding differences in diet between species, how dietary categories relate to herbivory in the wild, and how other attributes of biology, such as morphology and body size, relate to diet. Importantly this classification scheme also provides the means by which other measures, such as those used in DMTA, can be related to diet.

2.0.1. Statement of Authorship

Sam Arman designed the study, collated, and analysed the data used in this chapter, and wrote the manuscript.

Gavin Prideaux helped guide the focus and scale of the project and contributed to the discussion.

As quoted in Jackson and Vernes (2010), Kangaroo, portrait of an extraordinary marsupial*, Allen and Unwin

Dietary classification of extant kangaroos and their relatives (Marsupialia: Macropodoidea)

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2.1. Abstract

Kangaroos and kin (superfamily Macropodoidea) are the principal endemic vertebrate herbivores of Australia and the most diverse radiation of marsupial herbivores ever to have evolved. As is typical of other herbivore groups (e.g., bovids), dietary niches span fruit, fungi, dicot leaves and monocot grasses in both specialists and generalists, but to date dietary classification has been largely ad hoc and poorly tied to actual dietary ecological data. Here we provide a simple dietary classification of the Macropodoidea based on an extensive literature survey. Intake of four major foods–grasses, dicot leaves, fruits and seeds, and fungi–was assessed using proportional intake for 19 species and categorical (ranked intake) data for 37 species. Statistical comparisons with cluster and principal components analyses aligned species into four dietary groups. Members of the first group have diets that primarily consist of fungi and fruits. Relative proportions of grasses to dicot leaves separate the remaining species into browser (more than 70% dicots), grazer (more than 70% grasses) and mixed feeder groups. Comparison of our diet-based classification with a prevailing scheme based on dental morphology suggests that most species with what has traditionally been viewed as a 'browser-grade dentition' are actually mixed feeders. This suggests that either morphology and diet are not tightly linked, or that morphological differences between the dentitions of browsers and mixed feeders are subtle and have been overlooked. A positive correlation was found between body mass and average proportional intake of grass in the diet

of macropodoids. This parallels the situation found in bovids, as well as the percentage cut-off between dietary groups. These trends suggest that some underlying ecophysiological constraints may influence food choice in mammalian herbivores, providing useful pointers to the diets of extinct taxa.

2.2. Introduction

Macropodoids (families Hypsiprymnodontidae, Macropodidae) occupy all regions of mainland Australia, Tasmania and New Guinea, and in many ways, are the marsupial equivalent of artiodactyl ungulates. There are approximately 70 extant species (Van Dyck and Strahan 2008), ranging in size from the 0.5 kg musky rat-kangaroo (*Hypsiprymnodon moschatus*), the sole living representative of its family, to the large kangaroos of the genus *Macropus*, which can weigh up to 90 kg (Dawson 1995). As a rule, most smaller species consume low-fibre diets and inhabit better-vegetated habitats, whereas larger kangaroos graze more open habitats (Jarman 1984, Norbury *et al.* 1989, Van Dyck and Strahan 2008). But beyond broad generalizations, macropodoid dietary classification is ill defined at best and has not been reviewed for a quarter of a century. This is despite an expanding body of direct evidence of the diets of individual species. Here we synthesize all available macropodoid dietary data, employing a similar clustering approach to that used in a landmark study of bovids (Gagnon and Chew 2000), as well as more recent dietary analyses (Pineda-Munoz and Alroy 2014), to create an empirically based dietary classification scheme.

Dietary categories based on indirect dietary inference (Sanson 1978, 1980, 1989, Norbury *et al.* 1989) have been utilised by most macropodoid dietary ecology researchers over the past two decades to classify the dietary ecology of their study species (e.g., Jarman *et al.* 1991, Davis *et al.* 2008, van Eeden *et al.* 2011). Sanson's classification was founded primarily on an ecomorphological interpretation of dental attributes; that is, diet was inferred from the food that each species appeared morphologically adapted to consume, not actual diet. Sanson

(1978) recognized a ‘basal browsing grade’ dentition purportedly adapted for consuming low-fibre browse, and a ‘derived grazing grade’ adapted for consuming higher fibre grasses, as well as a small number of browser–grazer intermediates. Later, he added a ‘basal macropodoids and potoroines’ dental grade corresponding to a diet of fruit, fungi and invertebrates (Sanson 1989). Other important groundwork was laid by Jarman (1984) who recognized how interactions between other ecological factors (e.g., climate, predators) affected foraging strategies. Norbury *et al.* (1989) were the first to unite the existing disparate sources of ecological data on kangaroos. In this, diet was quantified as a single variable of ‘preferred food’, non-primary dietary items were given minimal consideration, and no dietary classification was made (Norbury *et al.* 1989). Significant morphological work has also been undertaken by Janis (1990) and more recently by others (e.g., Lentle *et al.* 1998, 2003, Warburton 2009, Lentle and Hume 2010, Butler *et al.* 2014). Although some comprehensive ecological studies of individual species have developed their own *ad hoc* dietary classification (e.g., Horsup and Marsh 1992, Di Stefano and Newell 2008, Tuft *et al.* 2011), there has been no attempt to extend these to other taxa. This may be explained by issues in data comparability between studies due to inherent uncertainty in most data sets, such as the effect of relative digestability of foods for faecal pellet studies (e.g., Vernes 1995, Wann and Bell 1997, Lapidge 2000). Nonetheless, the absence of a macropodoid dietary classification scheme makes it difficult to reliably compare diets of closely related species and consider diet across the group. Precisely the same need drove the development of the bovid dietary classification scheme (Gagnon and Chew 2000).

Body mass has long been considered a predictor of diet in mammalian herbivores (Demment and Van Soest 1985). A number of mechanisms have been proposed to explain this, such as the inverse relationship of body mass to metabolic rate, and a positive relationship with gut capacity (Demment and Van Soest 1985), although both of these factors have been shown

recently to be consistent in relation to body mass (Clauss *et al.* 2013, Müller *et al.* 2013, Steuer *et al.* 2014). Others have suggested that smaller herbivores require less absolute energy than larger herbivores, so they tend to focus on foods that are high in digestible protein and carbohydrates such as fruits and fungi (Jarman 1984, Norbury *et al.* 1989). These items however are usually rare and/or unevenly distributed, meaning that only smaller species are able to rely on them, theoretically leading these smaller species to lose the ability to digest more cellulose-rich foods over time (Clauss *et al.* 2013). At the opposite extreme, larger herbivores can subsist on larger quantities of high-fibre foods (Müller *et al.* 2013). Grasses are abundant and widespread, but generally have high cellulose content, making them difficult to digest (Sanson 2006). Species that consume mostly grasses do so with symbiotic bacteria and large stomachs to facilitate extended digestion intervals, with knock-on effects on body size (Demment and Van Soest 1985). In addition, most herbivore species supplement their diet to some degree with dicots. Variation in nutritional content among dicot taxa and among different parts of individual plants (e.g., leaves, stem) means that the relationship between dicot nutrient quality and body size is complicated, especially given marked interspecific differences in browser selectivity (e.g., Jarman 1974, Norbury *et al*. 1989, Gagnon and Chew 2000). A recent review considering these and other factors, including fibre and protein content, and levels of secondary metabolites, found that overall digestibility of herbage is by far the strongest correlate of body size, possibly regulated by intake rates (Clauss *et al*. 2013). Here we explore these functional links by investigating how well proportional intake of grasses and dicots correlates with body mass in macropodoids.

2.3. Methods

2.3.1. Data collection

Dietary data were obtained from published literature in the form of gut contents (e.g., Griffiths and Barker 1966, Hollis *et al*. 1986, Dawson *et al*. 2004), faeces (e.g., Storr 1964,

Tory *et al*. 1997, van Eeden *et al*. 2011), habitat studies (Taylor 1980), feeding trials (McArthur *et al*. 2000), plot clipping (Wahungu *et al.* 1999, Stirrat 2002), stable-isotopic studies (e.g., Horsup and Marsh 1992, Telfer and Bowman 2006, McMillan *et al*. 2010), feeding observations (Taylor 1980, Lundie-Jenkins *et al.* 1993) and indigenous knowledge (Burbidge *et al.* 1988, Telfer and Garde 2006). Dietary items were classified into four categories that could be readily distinguished in most studies: grass (including dicot forbs, which were lumped with grasses by some authors), browse (dicot leaves and stems), fruit/seeds, and fungi.

Reliable proportional dietary composition data, including those arising from samples taken at different times of the year and different habitats, were available for 19 macropodoid species (table 2.1, figure 2.1, Appendix S1). To account for dietary variability, each study, as well as each sample from separate seasons or sites within a study, was considered to be an individual record for comparison.

As a larger body of qualitative dietary information is available for most macropodoids, approximate proportional intake of the dietary item was scored using a five-level consumption scale indicating relative proportions (table 2.2). Where information regarding selectivity for or against food types was available (e.g., Kirkpatrick 1965, Dawson *et al*. 1992, Sprent and McArthur 2002), or studies were undertaken on cleared agricultural land (e.g., Griffiths and Barker 1966, Griffiths *et al*. 1974, Wilson 1991), these factors were taken into account by placing more weight on the former and less on the latter subjectively by the authors. Categorical data were scored for 37 species of macropodoids, covering all extant genera except the poorly known *Dorcopsis* and *Dorcopsulus* forest wallabies of New Guinea (Appendix S2).

Average body mass data were acquired from Van Dyck and Strahan (2008), included in (table 2.1). These body mass data are based upon averages and do not take into account known sexual dimorphism; however, the use of average data is necessitated to allow comparability with similarly averaged dietary data (although see Newsome 1980 for an investigation of diet and sex in *Macropus rufus*). Correlations between proportional intake of grasses and dicots to body mass were calculated using average proportional dietary composition data only.

Table 2.1: Proportional data of dietary intake of kangaroos. 'n' refers to the number of samples for each taxon. 'kg' refers to body mass taken from van Dyke & Strahan 2008.

Taxon	Grass	Browse	Fruits/Seeds	Fungi	n	kg
Bettongia gaimardi	0.0	8.4	24.0	67.6	7	1.7
Bettongia tropica	12.8	39.0	0.5	47.7	12	1.2
Potorous tridactylus	7.6	10.3	19.5	62.4	17	1.1
Lagorchestes hirsutus	67.0	19.0	14.4	0.0	8	1.6
Macropus agilis	99.5	0.0	0.5	0.0	8	15
Macropus antilopinus	85.3	14.7	0.0	0.0	6	27
Macropus dorsalis	92.7	7.3	0.0	0.0	23	10.1
Macropus fuliginosus	79.5	20.5	0.0	0.0	13	36.5
Macropus giganteus	90.8	8.5	0.3	0.0	15	40.8
Macropus robustus	87.8	11.1	0.8	0.0	22	25.4
Macropus rufogriseus	27.5	72.5	0.0	0.0	2	17.1
Macropus rufus	81.1	18.9	0.0	0.0	18	40
Onychogalea fraenata	53.6	46.3	0.0	0.0	16	5.8
Petrogale assimilis	59.2	41.2	0.0	0.0	6	4.5
Petrogale penicillata	61.9	38.7	0.0	0.0	9	7.1
Petrogale xanthopus	61.7	37.5	0.0	0.0	13	9
Setonix brachyurus	20.3	79.5	0.0	0.0	12	3
Thylogale stigmatica	47.0	53.0	0.0	0.0	2	4.6
Wallabia bicolor	52.4	39.5	0.0	7.9	14	15

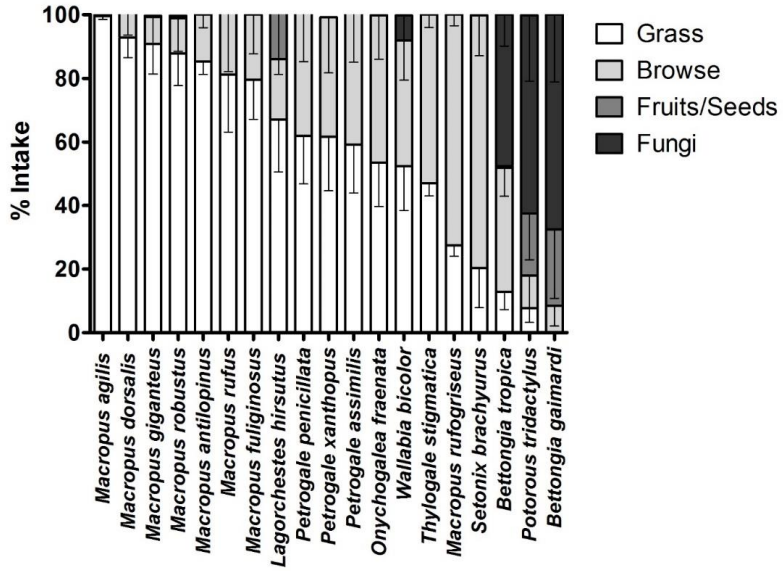

Figure 2.1: Mean ± 1 standard deviation of proportional dietary intake. See Appendix S1 for details.

Table 2.2: Consumption scale used to define dietary intake

Score	Proportion	Description	Percentage
5	Very High	Eaten in high quantities at all times	70–100%
4	High	Substantial dietary component at all times	30–69%
3	Moderate	Common dietary component eaten in some amount at all times	10–29%
2	Minor	Common but not substantial, may be seasonal	3–9%
1	Rare	Recorded but uncommon, may be seasonal	0–2%
0	Absent	Not documented	0%

2.3.2. Analysis

The collated data were non-normally distributed (Shapiro–Wilk test $P < 0.001$), and transformations were unsuccessful at normalising the data. Non-parametric Kruskal–Wallis tests were then used to determine if significant differences were present between species for each dietary item, and Bonferroni corrected Wilcoxon rank–sum comparisons were used to identify significant differences in diet between individual species. Mean and standard deviation of the proportional intake of each dietary item were then calculated for each species and analysed using cluster analysis. Paired group (Euclidean) algorithms were used, which

better distinguish large groups earlier in the analysis, and so are well suited for dietary clustering (Gagnon and Chew 2000). A problem with cluster analysis is that it will resolve clusters regardless of whether these clusters represent truly divergent groups (Hammer and Harper 2006). To respond to this, an alternate method of grouping species by diet was undertaken using principal components analysis (PCA), which reduces multivariate data sets into a smaller number of components representing the majority of variation to visualise differences between groups (Hammer and Harper 2006). As PCA is intended for use on multivariate normal data sets, a comparison was made between the scatter plot produced in PCA and those made in principal coordinates and discriminant function analyses, which do not have such properties. No differences were noted between these analyses so PCA was used as it allows greater investigation of the relative loadings of the components. Statistical tests could not be run on the categorical data, but cluster analysis and PCA were again used. Groups created in the statistical analysis, cluster analysis and PCA for both proportional and categorical data were defined along standard dietary categories and a final dietary categorisation reached by comparison of groupings across methods. Body mass data were log transformed prior to analysis to satisfy normality assumptions, and correlated to proportional intake of grass and browse using Pearson's *r*. The body size to diet relationship was further investigated through analysis of the residuals of a body mass to diet linear model. All analyses were conducted in PAST 2.16 (Hammer et al. 2001) and R (R Core Team 2014).

2.4. Results

2.4.1. Proportional data

Kruskal–Wallis comparisons indicated significant differences among species for all dietary categories (all $P \leq 0.001$). Significant Bonferroni corrected Wilcoxon rank–sum comparisons were variable across species (tables 2.3–2.4); however, similarities between a number of species reflect clear dietary groups, based on items comprising the core of their diet. Seven

species primarily grazing (*Macropus agilis, M. antilopinus, M. dorsalis, M. fuliginosus, M. giganteus, M. robustus* and *M. rufus*) were different from most other taxa in grass and, to a lesser extent, browse, but grouped with most other taxa for fruit/seeds and fungi. Mixed-feeding species (*Petrogale assimilis, P. penicillata, P. xanthopus, Onychogalea fraenata*, and *Wallabia bicolor*) were found to be distinct from other species in grass and browse, but not from fruit/seeds or fungi. *Lagorchestes hirsutus* showed similar trends to the grazers in grass and the mixed feeders in browse, possibly due to high seed consumption decreasing relative intake of other dietary items. *Setonix brachyurus* is likely the sole browser in this data set, and had different browse and grass consumption to grazers and mixed feeders, but not the fungivores. Fungivores (*Bettongia gaimardi, B. tropica*, and *Potorous tridactylus*) were distinct in fungi and to a lesser extent fruit/seed consumption. Both *Thylogale stigmatica* and *Macropus rufogriseus* could not be distinguished from any other species for any dietary item; however, both of these species had low sample sizes (each $n = 2$).

2.4.2. Average proportional data

The average proportional cluster analysis dendrogram (figure 2.2) identified four core dietary groups. The fungivore group removed at the first node was composed exclusively of potoroine species. Within the remaining taxa, *S. brachyurus* and *M. rufogriseus* were differentiated from all other taxa as browsing species. The final two core groups consist of seven species of small to medium-sized mixed-feeding species, and seven species of *Macropus* and *L. hirsutus* representing the grazing taxa.

Table 2.3 (page 48): P-values of interspecific dietary differences in Bonferroni corrected Wilcoxon rank–sum comparisons resulting from the Kruskal–Wallis test. Upper right is grasses; lower left is browse. Significant values ($P < 0.05$) in bold.

Table 2.4 (page 49): P-values of interspecific dietary differences in Bonferroni corrected Wilcoxon rank–sum comparisons resulting from the Kruskal–Wallis test. Upper right is fungi; lower left is fruit/seeds. Significant values ($P < 0.05$) in bold.

Table 2.3	*B. gaimardi*	*B. tropica*	*P. tridactylus*	*L. hirsutus*	*M. agilis*	*M. antilopinus*	*M. dorsalis*	*M. fuliginosus*	*M. giganteus*	*M. robustus*	*M. rufogriseus*	*M. rufus*	*O. fraenata*	*P. assimilis*	*P. penicillata*	*P. xanthopus*	*S. brachyurus*	*T. stigmatica*	*W. bicolor*
Bettongia gaimardi	-	0.054	0.107	0.136	0.087	0.244	**0.013**	**0.044**	**0.028**	**0.015**	1.000	**0.022**	**0.029**	0.244	0.105	**0.045**	**0.029**	1.000	**0.033**
Bettongia tropica	0.075	-	1.000	**0.042**	**0.035**	0.148	**0.000**	**0.004**	**0.002**	**0.000**	1.000	**0.001**	**0.002**	0.148	**0.024**	**0.004**	1.000	1.000	**0.010**
Potorous tridactylus	1.000	**0.016**	-	1.000	0.484	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**0.001**	0.375	0.240	**0.016**	0.133	1.000	**0.001**
Lagorchestes hirsutus	1.000	**0.042**	**0.013**	-	0.117	1.000	**0.042**	1.000	0.300	0.421	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Macropus agilis	0.089	**0.026**	**0.011**	0.069	-	0.268	0.257	**0.027**	0.274	**0.012**	1.000	**0.022**	**0.015**	0.268	0.082	**0.027**	**0.015**	1.000	**0.018**
Macropus antilopinus	1.000	0.149	0.061	1.000	0.131	-	1.000	1.000	1.000	1.000	1.000	1.000	0.103	0.842	1.000	1.000	1.000	1.000	0.090
Macropus dorsalis	1.000	**0.000**	**0.000**	0.126	0.074	1.000	-	0.144	1.000	1.000	1.000	1.000	**0.000**	**0.037**	**0.021**	**0.001**	**0.002**	1.000	**0.000**
Macropus fuliginosus	1.000	0.858	**0.001**	1.000	**0.021**	1.000	0.143	-	1.000	1.000	1.000	1.000	**0.023**	1.000	1.000	1.000	1.000	1.000	**0.027**
Macropus giganteus	1.000	**0.005**	**0.000**	0.663	0.320	1.000	1.000	0.626	-	1.000	1.000	1.000	**0.001**	0.172	0.103	**0.016**	**0.014**	1.000	**0.001**
Macropus robustus	1.000	**0.003**	**0.000**	1.000	**0.025**	1.000	1.000	1.000	1.000	-	1.000	1.000	**0.000**	0.161	0.147	**0.008**	**0.028**	1.000	**0.000**
Macropus rufogriseus	1.000	1.000	1.000	1.000	0.887	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Macropus rufus	1.000	0.364	**0.000**	1.000	**0.009**	1.000	1.000	1.000	1.000	1.000	1.000	-	**0.031**	1.000	1.000	1.000	0.078	1.000	**0.029**
Onychogalea fraenata	**0.045**	1.000	**0.000**	**0.046**	**0.013**	0.103	**0.000**	**0.025**	**0.001**	**0.000**	1.000	**0.031**	-	1.000	1.000	1.000	1.000	1.000	1.000
Petrogale assimilis	0.545	1.000	0.061	0.408	0.133	0.855	**0.036**	1.000	0.149	0.146	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000	1.000
Petrogale penicillata	0.801	1.000	**0.006**	1.000	0.053	1.000	**0.022**	1.000	0.101	0.107	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000
Petrogale xanthopus	0.144	1.000	**0.001**	1.000	**0.021**	1.000	**0.002**	1.000	**0.017**	**0.009**	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000
Setonix brachyurus	0.546	1.000	**0.042**	1.000	**0.013**	1.000	**0.004**	1.000	**0.022**	**0.024**	1.000	0.115	1.000	1.000	1.000	1.000	-	1.000	1.000
Thylogale stigmatica	1.000	1.000	1.000	1.000	0.887	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	1.000
Wallabia bicolor	0.053	1.000	**0.005**	0.082	**0.015**	0.157	**0.000**	0.394	**0.002**	**0.001**	1.000	0.122	1.000	1.000	1.000	1.000	1.000	1.000	-

Table 2.4	*B. gaimardi*	*B. tropica*	*P. tridactylus*	*L. hirsutus*	*M. agilis*	*M. antilopinus*	*M. dorsalis*	*M. fuliginosus*	*M. giganteus*	*M. robustus*	*M. rufogriseus*	*M. rufus*	*O. fraenata*	*P. assimilis*	*P. penicillata*	*P. xanthopus*	*S. brachyurus*	*T. stigmatica*	*W. bicolor*
Bettongia gaimardi	-	1.000	**0.045**	**0.045**	0.165	**0.000**	**0.002**	**0.000**	**0.000**	1.000	**0.000**	**0.000**	0.165	**0.024**	**0.002**	1.000	**0.003**	1.000	**0.017**
Bettongia tropica	**0.034**	-	**0.012**	**0.012**	0.056	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**	**0.000**	0.056	**0.006**	**0.000**	1.000	**0.000**	1.000	**0.001**
Potorous tridactylus	1.000	1.000	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.005**	1.000	-	0.738
Lagorchestes hirsutus	0.118	1.000	1.000	-	-	-	-	-	-	-	-	-	-	-	-	**0.005**	1.000	-	0.738
Macropus agilis	0.240	1.000	1.000	1.000	-	-	-	-	-	-	-	-	-	-	-	**0.029**	1.000	-	1.000
Macropus antilopinus	**0.000**	**0.031**	**0.049**	1.000	-	-	-	-	-	-	-	-	-	-	-	**0.000**	1.000	-	**0.003**
Macropus dorsalis	**0.003**	0.542	0.821	1.000	-	-	-	-	-	-	-	-	-	-	-	**0.000**	1.000	-	0.102
Macropus fuliginosus	**0.003**	1.000	1.000	1.000	1.000	1.000	1.000	-	-	-	-	-	-	-	-	**0.000**	1.000	-	**0.034**
Macropus giganteus	**0.001**	1.000	0.931	1.000	1.000	1.000	1.000	1.000	-	-	-	-	-	-	-	**0.000**	1.000	-	**0.004**
Macropus robustus	1.000	1.000	1.000	1.000	-	-	-	1.000	1.000	-	-	-	-	-	-	1.000	1.000	-	1.000
Macropus rufogriseus	**0.000**	0.126	0.196	1.000	-	-	-	1.000	1.000	-	-	-	-	-	-	**0.000**	1.000	-	**0.017**
Macropus rufus	**0.001**	0.224	0.345	1.000	-	-	-	1.000	1.000	-	-	-	-	-	-	**0.000**	1.000	-	**0.034**
Onychogalea fraenata	0.240	1.000	1.000	1.000	-	-	-	1.000	1.000	-	-	-	-	-	-	**0.029**	1.000	-	1.000
Petrogale assimilis	**0.035**	1.000	1.000	1.000	-	-	-	1.000	1.000	-	-	-	-	-	-	**0.002**	1.000	-	0.487
Petrogale penicillata	**0.003**	0.542	0.821	1.000	-	-	-	1.000	1.000	-	-	-	-	-	-	**0.000**	1.000	-	0.102
Petrogale xanthopus	1.000	**0.007**	1.000	0.073	0.085	**0.000**	**0.001**	**0.001**	**0.000**	1.000	**0.000**	**0.000**	0.085	**0.008**	**0.001**	-	**0.000**	1.000	**0.000**
Setonix brachyurus	**0.003**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**0.000**	-	1.000	0.388
Thylogale stigmatica	1.000	1.000	1.000	1.000	-	-	-	1.000	1.000	-	-	-	-	-	-	1.000	1.000	-	1.000
Wallabia bicolor	**0.001**	0.300	0.459	1.000	-	-	-	1.000	1.000	-	-	-	-	-	-	**0.000**	1.000	-	-

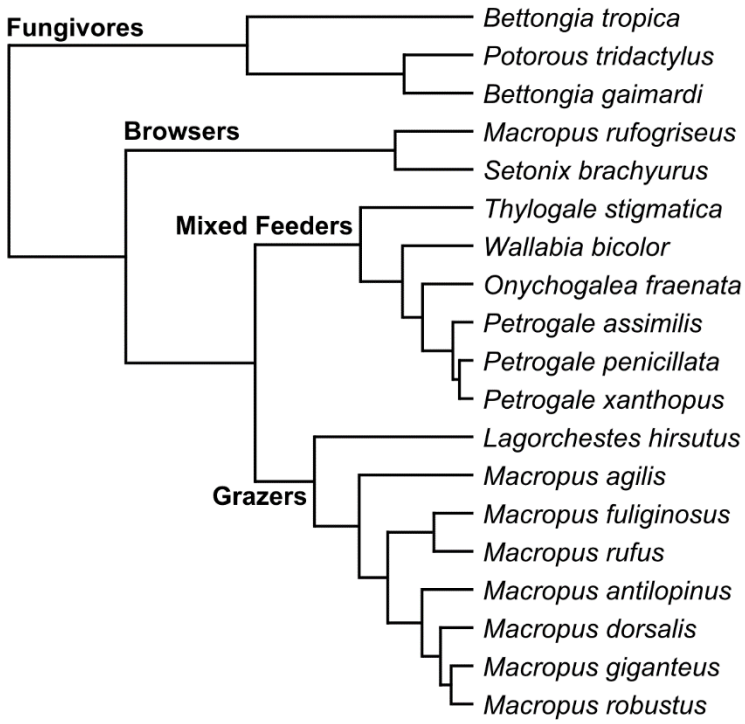

Figure 2.2: Dendrogram of the paired group (Euclidean) cluster analysis of the average proportional dietary data. Dietary groups (bold) are inferred based on shared dietary components.

The average proportional PCA scatter gram for the first two components (figure 2.3) showed a number of clearly distinct groups. On the upper-left aligned with high grass intake were eight grazing species (*M. agilis, M. antilopinus, M. dorsalis, M. fuliginosus, M. giganteus, M. robustus, M. rufus* and *L. hirsutus*). *Setonix brachyurus* and *M. rufogriseus* were again distinguished from most other taxa due to their high browse intake. A tight group of mixed-feeding species (*P. assimilis, P. penicillata, P. xanthopus, O. fraenata, T. stigmatica,* and *W. bicolor*) separate the predominantly browsing and grazing species. *Bettongia gaimardi* and *P. tridactylus* were grouped together as fungivorous taxa, although *B. tropica* appeared isolated between the fungivore and mixed-feeding and browsing taxa.

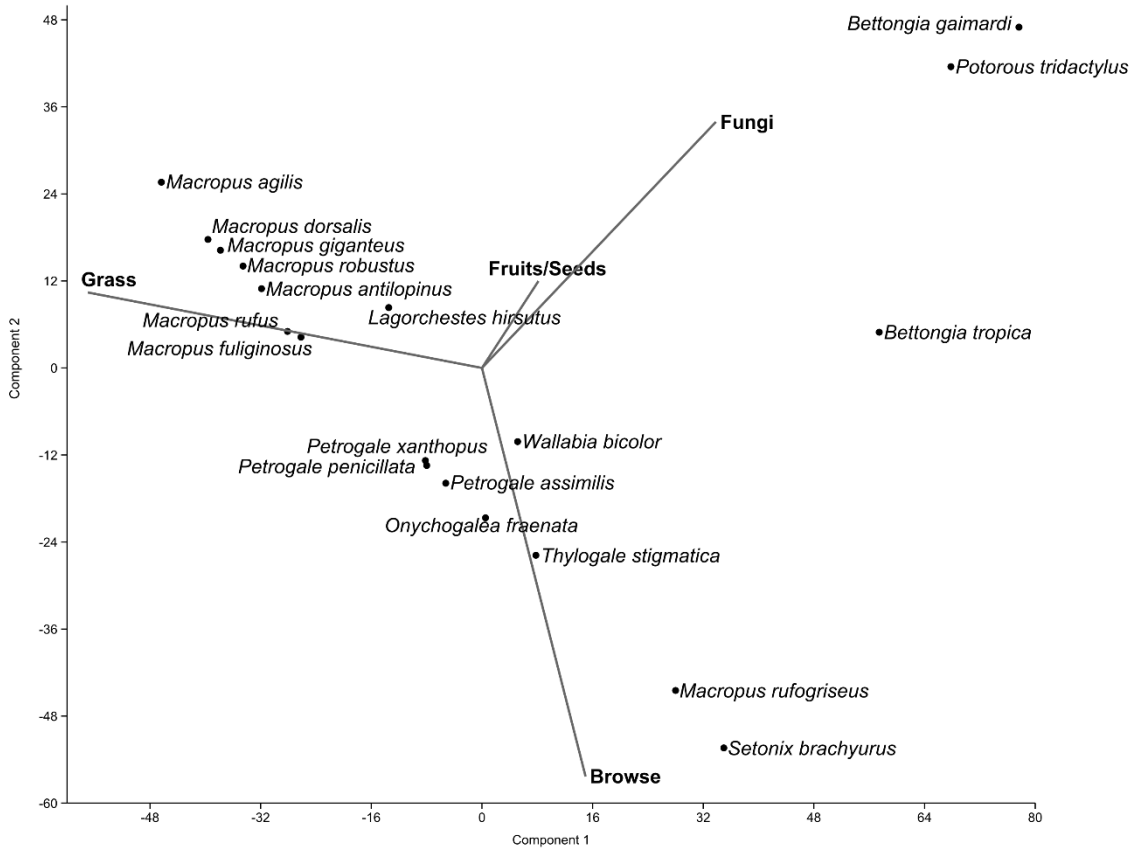

Figure 2.3: Scatter gram of components 1 (90% of variance) and 2 (4% of variance) of the principal components analysis of the average proportional dietary data. Relative loadings of the dietary items are also indicated.

2.4.3. Categorical data

The categorical cluster analysis indicated four core clusters in the data set (figure 2.4). The first node on the dendrogram separated the fungivore species from all other taxa, due to their moderate to very high consumption of fungi. The first node in the remaining group separated species consuming very high levels of browse from the remaining taxa. The next node separated a large group of mixed-feeding kangaroos from the grazers. The mixed-feeding kangaroos consume varying amounts of each of the dietary categories, but never very high amounts of any. The final group was composed of grazers that consume high to very high levels of grasses, no fungi and generally low but variable levels of fruit/seeds and browse.

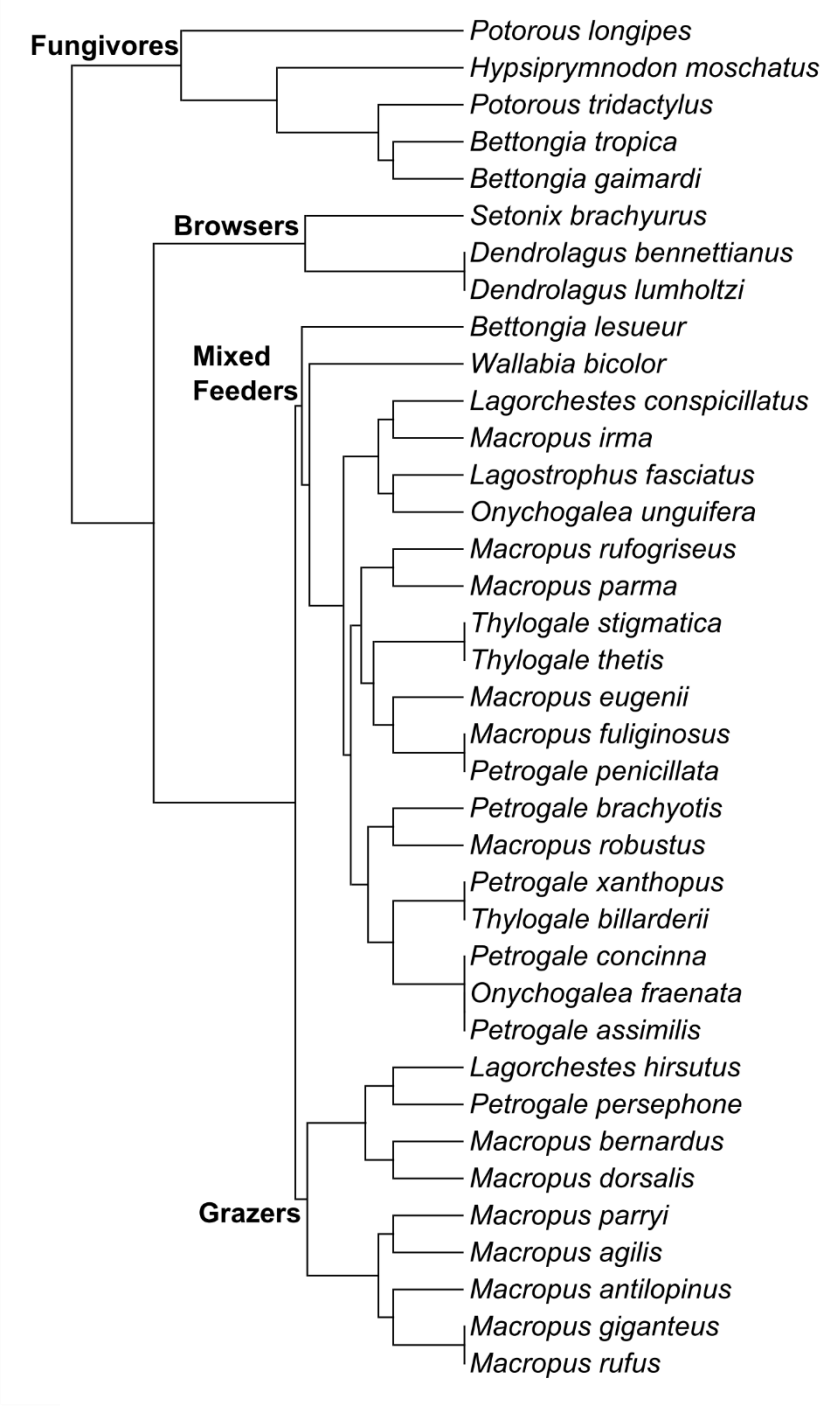

Figure 2.4: Dendrogram of the paired group (Euclidean) cluster analysis of the categorical dietary data. Dietary groups (bold) are inferred based on shared dietary components.

The scattergram of components 1 and 2 of the categorical PCA (figure 2.5) showed a large central cluster containing most species, with ancillary smaller groups that can be identified based largely upon very high intake of a few key dietary items. Four fungivore/frugivore

species (*P. tridactylus, B. tropica, B. gaimardi,* and *H. moschatus*) grouped together, and *P. longipes* groups with these in component one, but was distinguished in component two, likely due to this species consuming more browse and less fruits and seeds than other fungivores. Low in component 2, corresponding to high browse intake were the two *Dendrolagus* species and *S. brachyurus* as browsers, potentially with *Lagostrophus fasciatus*. Preference for grass or browse separated the remaining species along a continuum. Dividing these between mixed feeders and grazers was less clear; however, eight species (*L. hirsutus, M. agilis, M. bernardus, M. dorsalis, M. giganteus, M. parryi, M. rufogriseus,* and *M. rufus*) were grouped apart from the remaining taxa and have high grass consumption so best approximate grazers. The remaining taxa were considered mixed feeders.

2.4.4. Consensus

To combine the results generated from the different data and methods, results from each process were compared for each taxon, resulting in a final dietary consensus (table 2.5). In 30 of 37 species, the categorization was uniform across all methods that yielded meaningful results. *Macropus antilopinus, M. fuliginosus* and *M. robustus* were classified as grazers in most analyses, and as mixed feeders, in others, suggesting that these may be variable grazers that have a more mixed-feeding diet at times. Somewhat similarly, *L. hirsutus* was classified as a grazer in the cluster analyses and PCAs, but proved similar to both grazing and browsing taxa in the statistical analysis. A high proportion of seeds differentiates the diet of this species from the remaining grazers and mixed feeders. This may have lowered the proportional intake of both grass and browse, resulting in the contradictory classification. This specialization, perhaps unique within the Macropodoidea, makes *L. hirsutus* best considered as a seed-specialist grazer. *Potorous longipes* was grouped with the fungivores in the categorical cluster analysis, but between the fungivores and browsers in the categorical PCA, likely due to high consumption of browse and no fruit/seeds. Nonetheless, a very high intake

of fungi clearly indicates that this species is a fungivore. *Lagostrophus fasciatus* was grouped with mixed feeders in the categorical cluster analysis, but between the mixed feeders and the browsers in the categorical PCA, and may be best considered a mixed feeder. *Petrogale persephone* was classified as a grazer in the categorical cluster analysis, but a mixed feeder in the categorical PCA. Based on the raw data, *P. persephone* appears more closely allied with mixed feeders, but may have grouped with the grazers in the categorical cluster analysis due to having fruit/seeds as a minor dietary component, because fruit/seeds were only a variable component in mixed feeder diets but were almost always present in grazer diets. *Macropus rufogriseus* could not be distinguished from any other taxa in the statistical comparisons, and grouped with the browsing taxa in the mean proportional intake cluster analysis and PCA. It was considered a mixed feeder in the categorical cluster analysis, and a grazer in the categorical PCA. These contrasting conclusions are in part due to the only proportional data available for this species being from alpine regions of Tasmania where grass is scarce and browse abundant. Other records, however, support a mixed-feeding diet for this species, with rare consumption of fruits and seeds again potentially aligning this species with grazers over mixed feeders in the categorical analyses.

2.4.5. Body size

Log body mass and proportional grass intake were correlated ($r = 0.751$, $P \leq 0.001$, figure 2.6); however, log body mass to browse could not be similarly correlated ($r = -0.191$, $P = 0.192$). Residual values from a linear model of the proportioinal grass intake–log body mass relationship (figure 2.6, Appendix S1) show the greatest deviation in a small number of taxa. For *B. gaimardi* and *L. hirsutus*, this is best explained by their diet being supplemented by other energy-rich foods, in fungi and seeds respectively. For *M. rufogriseus* the source of dietary data may be problematic (see above). Two other species with high residual values,

M. agilis and *M. dorsalis*, are less clear, and these species appear to be consuming much higher proportions of grass than their body mass would predict.

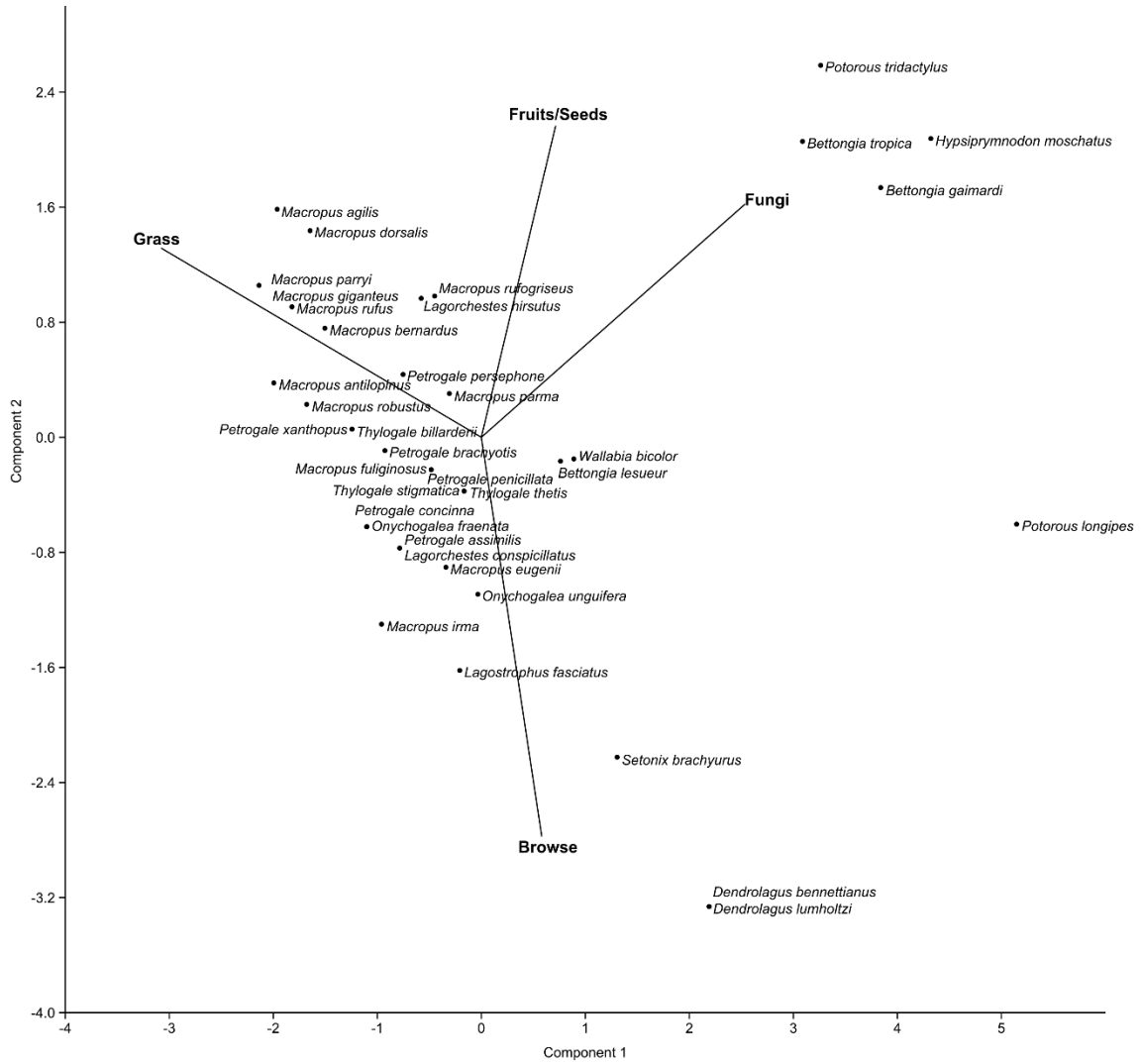

Figure 2.5: Scattergram of components 1 (57% of variance) and 2 (27% of variance) of the principal components analysis of the categorical dietary data. Relative loadings of the dietary items are also indicated.

Table 2.5: Summary of results and dietary consensus reached. B, browser; CA, cluster analysis; F, fungivore; G, grazer; K–W post hoc, Bonferroni corrected Wilcoxon rank–sum comparisons resulting from the Kruskal–Wallis test; MF, mixed feeder; PCA, principal components analysis.

	Full Dataset	Mean Proportional		Categorical		Consensus
	K–W posthoc	CA	PCA	CA	PCA	
Hypsiprymnodon moschatus				F	F	Fungivore
Bettongia lesueur				MF	MF	Mixed Feeder
Bettongia gaimardi	F	F	F	F	F	Fungivore
Bettongia tropica	F	F	F	F	F	Fungivore
Potorous longipes				F	F/B	Fungivore
Potorous tridactylus	F	F	F	F	F	Fungivore
Dendrolagus bennettianus				B	B	Browser
Dendrolagus lumholtzi				B	B	Browser
Lagorchestes conspicillatus				MF	MF	Mixed Feeder
Lagorchestes hirsutus	G/MF	G	G	G	G	Seed Specialist
Lagostrophus fasciatus				MF	B/MF	Mixed Feeder
Macropus agilis	G	G	G	G	G	Grazer
Macropus antilopinus	G	G	G	G	MF	Grazer
Macropus bernardus				G	G	Grazer
Macropus dorsalis	G	G	G	G	G	Grazer
Macropus eugenii				MF	MF	Mixed Feeder
Macropus fuliginosus	G	G	G	MF	MF	Grazer
Macropus giganteus	G	G	G	G	G	Grazer
Macropus irma				MF	MF	Mixed Feeder
Macropus parma				MF	MF	Mixed Feeder
Macropus parryi				G	G	Grazer
Macropus robustus	G	G	G	MF	MF	Grazer
Macropus rufogriseus	Unclear	B	B	MF	G	Mixed Feeder
Macropus rufus	G	G	G	G	G	Grazer
Onychogalea fraenata	MF	MF	MF	MF	MF	Mixed Feeder
Onychogalea unguifera				MF	MF	Mixed Feeder
Petrogale assimilis	MF	MF	MF	MF	MF	Mixed Feeder
Petrogale brachyotis				MF	MF	Mixed Feeder
Petrogale concinna				MF	MF	Mixed Feeder
Petrogale penicillata	MF	MF	MF	MF	MF	Mixed Feeder
Petrogale persephone				G	MF	Mixed Feeder
Petrogale xanthopus	MF	MF	MF	MF	MF	Mixed Feeder
Setonix brachyurus	B	B	B	B	B	Browser
Thylogale billarderii				MF	MF	Mixed Feeder
Thylogale stigmatica	Unclear	MF	MF	MF	MF	Mixed Feeder
Thylogale thetis				MF	MF	Mixed Feeder
Wallabia bicolor	MF	MF	MF	MF	MF	Mixed Feeder

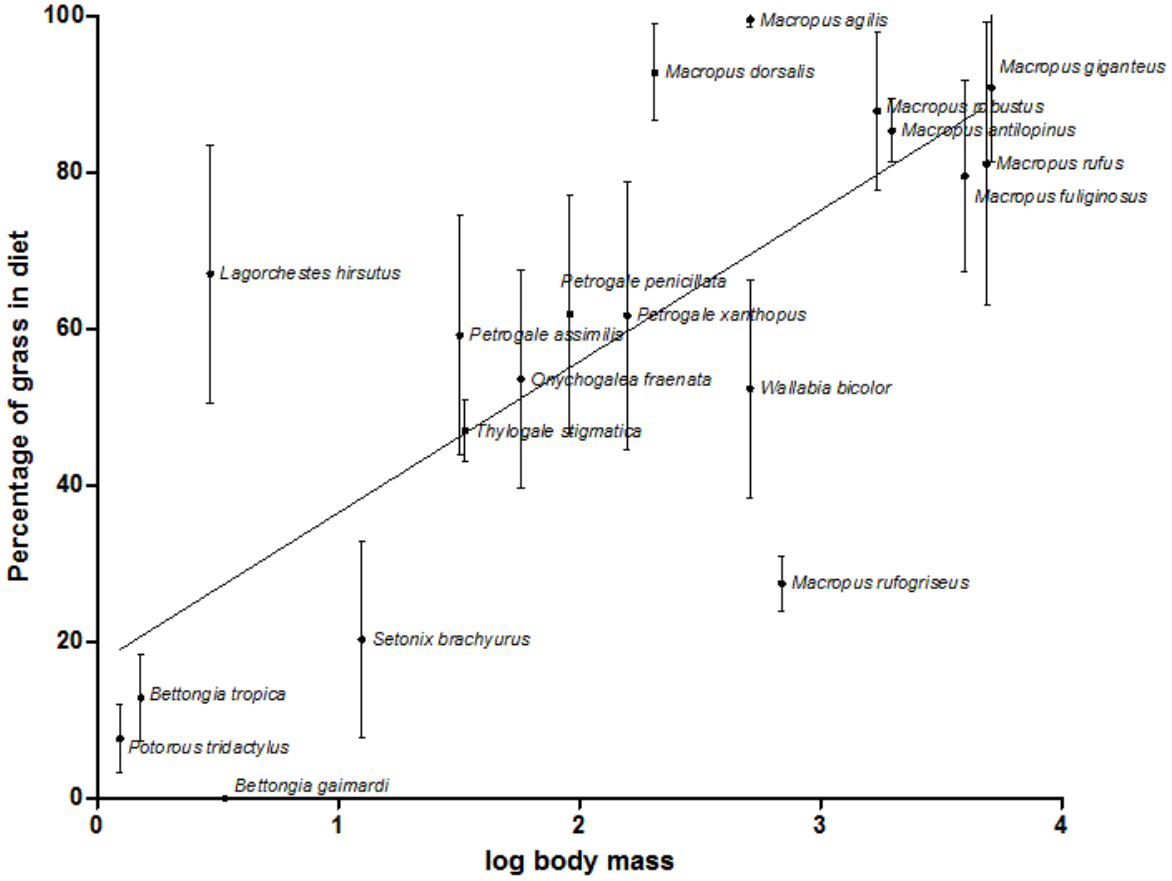

Figure 2.6: Correlation of log average body mass to average proportional grass intake ± 1 standard deviation.

2.5. Discussion

2.5.1. Dietary classification

When dietary classification is compared against average proportional intake of the four dietary categories, the defining features of each dietary group is evident. Fungivores all had fungi as more than 40% of their diet on average, often with a high proportion of fruit. The proportion of grass to browse separated the remaining species. Browsers consume less than 30% grass and more than 70% browse on average. Mixed feeders consumed 30–70% of both grass and browse on average, generally with a higher proportion of grass. Grazers consume more than 70% grass and less than 30% browse. In contrast, the proportional intake of grass to browse (figure 2.1) forms a continuum across all non-fungivorous species, largely

irrespective of dietary groups. The question then is whether this dietary classification is being artificially placed on a continuous dietary spectrum. The 70:30 split in grazers and browsers corresponds closely to that recognized for bovids, where browsers also consumed less than 70% browse and more than 30% grass, although grazers were split into variable grazers (60–90% grass) and obligate grazers (more than 90% grass) (Gagnon and Chew 2000).

The similarity in dietary niche between bovids and macropodoids suggests that a consistent dietary niche pattern may emerge for all mammalian herbivore radiations in similar environments. Moreover, the consistent change in dietary niche near this 70% intake of a single dietary item may represent a tipping point in dietary specialisation where consuming a single type of food becomes more advantageous than retaining a more mixed diet. The precise mechanism for this is unclear, but may be related to the specialisation of gut flora to process a particular food type, as has been seen for coarser dietary differences elsewhere (Ley *et al.* 2008). Indeed, differences in oesophageal bacteria between macropodoid species (Obendorf 1984) largely follow dietary groups defined here. Most dietary groups recognised were also comparable with those found in bovids (Gagnon and Chew 2000), with the exception of the bovid frugivore group, which consumes more than 70% fruits (Gagnon and Chew 2000). No such group was identified for macropodoids, and only a few taxa, notably the rainforest specialist *H. moschatus* consume a high proportion of fruits. This may be related to the competitive presence of frugivorous phalangerids (possums) in Australia (Van Dyck and Strahan 2008), although similar frugivorous competitors (e.g., primates, rodents) also exist in bovid habitats. In contrast, Gagnon and Chew (2000) did not recognize a fungivorous group, with this niche likely being occupied by other mammals such as sciurids (squirrels and chipmunks) (see Fogel and Trappe 1978). Indeed, potoroines are more similar to sciurids than bovids in their capacity to dig and manipulate foods with their forelimbs (Jarman 1984, McDowell *et al*. 2015). The proportion of species in each dietary group is also

different; there are more mixed-feeding macropodoids, but more grazing bovids. This is potentially related to biogeography, broad biological differences between these herbivores, or alternatively taxonomic factors, such as the high number (six species) of mixed-feeding rock wallabies (*Petrogale*).

A small number of taxa investigated here appear to have unexpected diets that warrant further discussion. The unambiguous placement of *Bettongia lesueur* in the mixed-feeding group rather than the fungivorous group, which includes all other potoroines for which dietary data exist, is unexpected. Recent data, however, are quite clear in demonstrating that *B. lesueur* has a high intake of browse (Robley *et al.* 2001). *Bettongia lesueur* and the recently extinct *B. anhydra* from arid Australia have morphological features that are consistent with an ability to consume tougher food items than their temperate cousins (McDowell *et al.* 2015). Sanson (1978, 1980, 1989) considered *W. bicolor* to express the quintessential browser-grade dental morphology, with its flat molar row and blade-like premolar, but numerous studies clearly indicate that it is a mixed feeder (e.g., Hollis *et al.* 1986, Osawa 1990, Di Stefano and Newell 2008).

2.5.2. Dental morphology

Overall, most fungivorous, browsing and grazing taxa consume foods that they appear morphologically adapted to eat. However, numerous taxa identified here as mixed feeders were considered by Sanson (1978, 1980, 1989) to have a browser-grade morphology. This disparity may result from a preadaptation of mixed feeders to a browser-grade dentition while actually consuming a broader range of foods. This idea is not new. In reference to *T. stigmatica*, Vernes (1995) argued that a browser-grade morphology simply allowed for 'dietary latitude' in these species. The ability of animals to process foods with lower energy extracted per unit effort (i.e., low energy/effort grass vs. high energy/effort browse), 'fallback feeding', becomes advantageous when preferred foods are unavailable and is considered an

adaptation to seasonal or unpredictable environments (Constantino and Wright 2009). Rather than falling back to less palatable grasses as seen in other groups, kangaroos with a mixed-feeding diet but a grazer-grade dentition appear to be 'falling forward' to the more palatable browse. Browser-grade dental morphology in these species may alternatively indicate a recent switch from browsing to mixed-feeding, without sufficient time elapsed to allow an adaptive shift in dentition. This prompts the question as to what adaptations may characterise the dentition of a mixed feeder, a group to which little attention has thus far been paid. Sanson (1978, 1989) considered browser- and grazer-grade dentitions to be the 'adaptive peaks' in morphology, with the few intermediates recognised as being in an evolutionary flux of sorts as they traverse the morphospace between. Rather than categorisation however, Jarman (1984) focused on mixed feeding as an inherent flexibility in kangaroo diets, as a result of seasonality, landscape heterogeneity and ecological trade-offs with other activities. Considering the abundance of mixed-feeding species, it may be that the browser-grade morphology recognized by Sanson (1978, 1980, 1989) is in fact a mixed-feeding morphology, or that there are more subtle distinctions between browser-grade and mixed-feeder-grade morphologies yet to be identified.

One possible distinction in dental morphology between dietary groups may be crown height, with species identified as browsers by Janis (1990) having lower crown height than both mixed feeders and grazers. Further increases in crown height as seen in ungulates may however be limited by the macropodoid bilophodont condition (Janis 1990). Alternately, some differences may be functional, such as the ability of ungulates to re-chew regurgitated foods while kangaroos must complete particle size reduction before swallowing. Molar progression has also been considered to be an adaptation to grazing (e.g., Sanson 1978, 1989, Janis 1990). However when body size is taken into account, the rate of molar progression is comparable between macropodoid species, with the most dramatic results only evident in

longer lived larger grazers (Lentle and Hume 2010). Enamel thickness may also play a role as has been documented along dietary lines in chiropterans and primates (Dumont 1995). The only comparative study on macropodoids showed little difference in enamel microstructure between the grazer-grade *M. giganteus*, mixed-feeder-grade *P. concinna* and browser-grade *W. bicolor*, but showed some differences in enamel hardness and thickness (Palamara *et al*. 1984). In particular, *M. giganteus* had harder enamel than *P. concinna* and *W. bicolor*, and differences were greatest in the outer occlusal surface rather than inner enamel (Palamara *et al*. 1984).

Previous work on equids suggests that functional morphology can only distinguish browsers and grazers, but not mixed feeders (Janis 1995). Certainly, the browser-grade macropodoid morphology appears well suited to feeding on both grass and browse, as is the norm, and the ability to process browse as the core of the diet may have just been taken to the extreme by the few species that are in the rare environmental conditions where browse can provide most of their nutritional requirements. Indeed, the distribution of browsers in Africa has been shown to be associated primarily with habitat type, which plays a greater role on distribution than other factors such as rainfall, nutrient status, latitude, primary productivity and biogeography (McNaughton and Georgiadis 1986). Moreover, browser-grade macropodoids have relatively narrower occlusal contacts on the incisors than grazer-grade species, which facilitates selective feeding (Sanson 1989), although Janis (1990) found muzzle width to be significantly wider in browser-grade kangaroos than other groups. Narrower occlusal contacts allow increased selective feeding on the most digestible browse compared with grazer-grade mixed feeders. The digestibility of plant structural types also relates to their permanency (Demment and Van Soest 1985). This means that by selectively feeding, browser-grade mixed feeders should be more likely to consume easily replaceable plant structures, causing less damage to plants than grazer-grade species and promoting regrowth. This may be an

important feedback mechanism in establishing and maintaining ecosystems through plant–herbivore interactions.

By highlighting that most supposed browser-grade macropodoids are in fact mixed feeders, one might ask: where are Australia's endemic large browsers? There still appears to be a considerable amount of potential browse vegetation, evinced by the number of mixed feeders that consume browse to varying degrees, as well as other browsing taxa such as possums and emus. In addition, the success of feral camels and deer in Australia has been largely attributed to their ability to utilise native trees and shrubs rarely touched by extant macropodoids (Duncan 1992, Edwards *et al.* 2010). Some of these plants retain now-obsolete physical and chemical defences against predation, or have fruit adapted for dispersal by large browsers (Murray and Vickers-Rich 2004, Johnson 2006). In all probability, the now-empty browsing niches were filled by sthenurine kangaroos, which made up 40% of the species lost from Australia during the late Pleistocene (Prideaux 2004). The morphological evidence for sthenurines as browsers goes well beyond teeth. Many skeletal attributes not observed in browser-grade macropodines (e.g., robust shortened skull, vertically oriented masticatory muscles, elongated, highly mobile forelimbs) point to sthenurines as the missing macropodoid browsers (Wells and Tedford 1995, Johnson and Prideaux 2004, Prideaux 2004). Other large taxa that became extinct during the late Pleistocene may also at least partly account for the vacant large browser niches (e.g., the giant flightless bird *Genyornis newtoni*, and quadrupedal diprotodontids *Diprotodon optatum* and *Zygomaturus trilobus*), but sthenurines likely made up the majority.

2.5.3. Gut morphology and body size

Much of the discussion on morphology in relation to diet focuses on the physical properties of foods, and the dental apparatus, but dietary adaptations also occur further down the digestive tract. Fungivorous taxa have larger sacciform forestomachs, while the tubiform

forestomach is better developed in large grazing kangaroos (Hume 1999). Haustration, an infolding of the forestomach, is developed to the greatest degree within the Macropodoidea in the tree kangaroos (*Dendrolagus*), which are browsers (Hume 1999). Haustration and associated wave-like muscular contractions are thought to assist in mixing of foods. These contractions have only been minimally studied, but action potentials indicative of stomach contractions associated with mixing of digesta were recognised in the browsing *Setonix*, but not the mixed-feeding *Thylogale* (Hume 1999), so they may play a role in assisting to digest tough browse by mixing this material with more palatable foods. Stomachs of grazing and mixed-feeding taxa are more typical of macropodoids, with a long foregut to extend microbial fermentation (Hume 1999). Again, these adaptations are analogous to but distinct from those seen in bovids (Gagnon and Chew 2000), and further indicate how diet can be used as a predictive tool in understanding comparative physiology.

The correlation between grass intake and body size echos results found elsewhere (e.g., Demment and Van Soest 1985, Gagnon and Chew 2000, Clauss *et al.* 2013). However, fundamental differences between macropodoids and bovids in gut bacterial flora (Ouwerkerk *et al.* 2005), stomach morphology and metabolic rates (Hume 1999) have the potential to affect comparisons of mass to diet. Log body mass was shown to correlate to grass intake (figure 2.6). This most likely reflects grasses being the most abundant food source in Australia and so increasingly relied upon by larger species. While the overall correlation between mass and grass consumption is comparable, diets between similarly sized macropodoids and bovids are quite different. For example, the macropodoid grazers identified here range from approximately 10 to 40 kg on average. Species falling into that range in the bovid analysis of Gagnon and Chew (2000), were primarily frugivores ($n = 4$) or browsers ($n = 3$), and only two were considered grazers. This relates to the overall differences in size range of these herbivores, where kangaroos range from 0.5 to 41 kg on average,

whereas bovids range from 3 to 544 kg on average. That the correspondence between mass and diet was independent of absolute mass suggests that although diet may play a key role in relative body mass of related species, coarse differences between bovids and macropodoids are related to other features of their core biology, life history and evolution. The analysis of residuals of the proportional grass–log body mass model showed that the most significant deviations from this relationship were those supplementing their diet with other energy-rich items. A number of grazing taxa whose diets differed from that which would be predicted based on their mass show that other factors, such as dietary niche partitioning may be influencing the relationship.

The seemingly proportional intake of browse (presuming browse intake is inversely proportional to grass intake) to inverse body mass did not correlate. This contrasts with the bovid evidence, where browse intake is negatively correlated with body mass (Gagnon and Chew 2000), although this is partly due to the two largest browsers in the African landscape, giraffes and elephants, being non-bovid. The lack of correlation of body mass to browse in macropodoids may further reflect the lack of extant browsers. However, as most sthenurines were heavier than macropodine kangaroos (Helgen *et al*. 2006), it is unlikely that the inclusion of these species would assist in correlating body mass to browse intake. Another explanation may be that the patchy distribution of browse combined with an abundance of mixed feeders simply outweighs any broad trends.

2.5.4. Future work

This analysis of existing data provides the most comprehensive dietary classification of the Macropodoidea to date. Although definitive for some species, dietary classification for many is based on limited data, and only by collecting more data for these species can their diets be determined. That said, however, the average proportional intake for dietary groups provided here should allow for simple dietary classification. Many other species of kangaroos were

also present in the relatively recent geological past, most importantly sthenurines. As these covered a broad size range (Prideaux 2004), it is tempting to apply the body size to grass intake correlation to these species to determine diet. Based on morphology, however, these species are considered universally to have been browsers or mixed feeders (e.g., Raven and Gregory 1946, Wells and Tedford 1995, Prideaux 2004). Current diversity in extant macropodoid diets may have evolved in response to the extinction of the sthenurines, which resulted in increased browse being available to macropodines. Looking at diet through the Neogene, in conjunction with morphology and body size will help explain kangaroo and herbivore dietary evolution. It should further illuminate how biogeographic history coincides with generalised adaptive mechanisms to shape species body size and dental morphology, whereas diet itself is adjusted over shorter timeframes in response to local environmental conditions and food availability.

2.6. Acknowledgements

Thanks to Matt McDowell, Trevor Worthy and Rod Wells for looking at drafts, and to Karl Vernes, Stuart Green, Naomi Davis, Melissa Danks, Matt Hayward and David Ramsey for providing unpublished data. Financial support was provided by a Flinders University Research Scholarship to SDA and an Australian Research Council grant (DP110100726) to GJP. We are grateful to Marcus Clauss, one anonymous reviewer and the associate editor for their useful criticisms.

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Chapter 3

Minimizing inter-microscope variability in Dental Microwear Texture Analysis.

'A man with one watch knows what time it is; a man with two is never sure'

-Segal's Law*

3.0. Context

DMTA data for this thesis was collected in the early stages on 'Connie', a confocal profiler housed at the University of Arkansas. An ARC LIEF grant awarded to Gavin Prideaux *et al.* in 2012 led to the purchase of a newer profiler, 'Bruce' now housed at Flinders University, where the remaining DMTA data were collected. During this process however, it was noted that substantial differences were evident between data collected on different profilers. Understanding and then mitigating these differences then led to this chapter.

3.0.1. Statement of Authorship

Sam Arman designed the study, collected, and analysed the data used in this chapter, and wrote the manuscript.

Peter Ungar provided access to the 'Connie' and 'Wall-E' profilers at the University of Arkansas, helped guide the research and contributed to the discussion.

Christopher Brown provided access to the 'Zeus' and 'Persephone' profilers at Worchester Polytechnic Institute, and provided technical feedback.

Larisa DeSantis provided access to the 'Dolly' profiler at Vanderbilt University.

Christopher Schmidt provided access to the 'Indie' profiler at the University of Indianapolis.

Gavin Prideaux helped guide the project and contributed to the discussion.

*Attributed to Lee Segal, but appears to have earlier origins. See:

http://www.barrypopik.com/index.php/new_york_city/entry/a_man_with_one_watch_knows_what_time_it_is_a_man_with_two_is_never_sure

Minimizing inter-microscope variability in dental microwear texture analysis

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Previously published as Arman, S. D., P. S. Ungar, C. A. Brown, L. R. DeSantis, C. Schmidt and G. J. Prideaux (2016). Minimizing inter-microscope variability in Dental Microwear Texture Analysis. *Surface Topography: Metrology and Properties* **4,** 024007

3.1. Abstract

A common approach to Dental Microwear Texture Analysis (DMTA) uses confocal profilometry in concert with scale-sensitive fractal analysis to help understand the diets of extinct mammals. One of the main benefits of DMTA over other methods is the repeatable, objective manner of data collection. This repeatability, however, is threatened by variation in results of DMTA of the same dental surfaces scanned by different profilers. Here we compare DMTA data of five species of kangaroos measured on seven profilers of varying

specifications. Comparison between microscopes confirms that inter-microscope differences are present, but we show that deployment of a number of automated treatments to remove measurement noise can help minimise inter-microscope differences. Applying these same treatments to a published hominin DMTA dataset shows that they alter some apparent significant differences between dietary groups. Minimising microscope variability while maintaining interspecific dietary differences requires then that these factors are balanced in determining appropriate treatments. The process outlined here offers a solution for allowing comparison of data between microscopes, which is essential for ongoing DMTA research. In addition, the process undertaken, including considerations of other elements of DMTA protocols also promises to streamline methodology, remove measurement noise and in doing so, optimise recovery of a reliable dietary signature.

3.2. Introduction

Dental microwear is the study of microscopic features that form on teeth through the impact of foods. Documenting microwear of specimens for which the diet is known quantifies how microwear relates to diet, and comparing these to microwear of extinct taxa allows their diets to be inferred. Although initially limited to qualitative studies based on binocular light microscopy (e.g., Simpson 1925, Butler 1952, Baker *et al*. 1959, Mills 1963) and then scanning electron microscopy (e.g., Baker *et al.* 1959, Walker et al 1978, Teaford 1985, Ungar and Grine 1991), researchers began quantifying microwear patterns by measuring scratches and pits on SEM photomicrographs in the 1980s (e.g., Grine 1984, Teaford and Walker 1984). Such studies were plagued by high observer-error rates due to information lost when reducing 3D surfaces to 2D, and measurement of hundreds of features with irregular borders (Grine *et al*. 2002; Galbany *et al*. 2005). These limitations led some researchers to develop alternative means, ultimately leading to Dental Microwear Texture Analysis (DMTA) (Ungar *et al.* 2003). DMTA uses confocal microscopy to collect 3D surface scans

that are analysed using a collection of algorithms known collectively as scale-sensitive fractal analysis (SSFA) (e.g., Ungar *et al*. 2006, 2012, Ungar 2011) or Surface Texture Analysis (STA) based on International Organization for Standardization (ISO) algorithms (e.g., Kaiser and Wolff 2005, Purnell *et al*. 2013, Goodall *et al*. 2015).

Much of the appeal of DMTA was its repeatable, objective nature, which promised to remove observer measurement-error effects and simplify comparisons made between data generated by different researchers. Recently, however, differences have been found between newer and older confocal profilers (Ungar *et al*. 2014), which bring into question the reliability of comparisons between different profilers, and the overall repeatability of the methodology when comparing datasets collected using different instruments. Similar considerations have led metrologists to compare instruments, but these studies focused on differences between different types of instruments and typically use standardized fabricated surfaces (e.g., Sandison *et al*. 1995, Dixson and Orji 2007, Vorburger *et al*. 2007). Rarer still are microscope comparisons using biological structures, which have been limited to qualitative not quantitative differences (White *et al*. 1987).

Surfaces often vary at different scales, as the phenomena that create the features act on different scales (Whitehouse 1994). For instance, the coarse shape of a tooth is involved in tooth–tooth interactions in guiding the occlusal cycle, however at finer scales the shape of the facet on the tooth reflects food–tooth interactions to break down food (Ungar 2010). Even within a facet or a single DMTA scan, dental surfaces vary at different scales. The smallest dental microwear features commonly measured are ~1 μm across (Nystrom *et al*. 2004), and differences between species have been observed at the scale of 0.1 μm (Teaford and Walker 1984). On a scan that generally has an area less than 1000 μm^2, however, these dimensions make even the smallest microwear features relatively large. In contrast, finer-scale analysis looks at features at scales as low as 0.01 μm, which may be influenced by a range of factors

including molding material, enamel microstructure, and measurement noise. To help parse such phenomena, and focus on scales of interest, metrologists often use filters to separate surface properties between coarse scale 'waviness' and fine scale 'roughness' (Whitehouse 1994).

This study considers inter-microscope differences by scanning, to the extent that is possible, identical tooth surfaces on a range of instruments. For the first time, a rigorous statistical approach is applied to comparisons. In addition, serial scanning on each machine helps quantify noise present in confocal profilometry of microwear surfaces. Finally, we assess effects of filters and other surface modifications to minimise noise and maximise the signal available for DMTA, and test the applicability of these by utilising the same filters on an existent DMTA dataset.

3.3. Methods

3.3.1. Institutional abbreviations

Specimens used in this study are housed in the: Australian Museum, Sydney (prefix AM M); Flinders University Research Collection, Adelaide (FU); Queensland Museum, Brisbane (QM A, J, JM), South Australian Museum, Adelaide (SAM M, P, FU).

3.3.2 Data collection

Specimens were cleaned and replicated using standard procedures (Scott *et al.* 2006), which have recently been shown to be precise and accurate at replicating the original surface (Goodall *et al.* 2015). Five specimens each of five species of macropodids (kangaroos) were used. Species chosen were *Dendrolagus lumholtzi* (Lumholtz's Tree-Kangaroo), *Macropus dorsalis* (Black-striped Wallaby), *Macropus rufus* (Red Kangaroo), *Wallabia bicolor* (Swamp Wallaby) and *Simosthenurus occidentalis* (an extinct short-faced kangaroo). These were chosen because together they cover the continuum of grazing (grass eating) to browsing

(dicot leaf eating) in kangaroos (Arman and Prideaux 2015), and so should present typical microwear variability.

Scanning was conducted on confocal profilers of varying specifications (table 3.1), with scanning hue used to measure topography in all cases. 'Connie' is the most senior profiler, and the majority of published DMTA data has been generated on this microscope. 'Indie' is a slightly newer device of similar specifications. The three NEOX Plμ profilers ('Bruce', 'Dolly' and 'Wall-E') are newer still, and allowed comparison of blue and white light. 'Persephone' is a replacement instrument for 'Zeus' using the same lens, but of a distinct make to the other profilers. During initial scanning on Bruce, a series of screenshots and notes were used to assist in re-locating the same location within the facet on other microscopes.

Table 3.1: Specifications of microscopes used in this study. Spatial sampling represents the x–y size in micrometres of each point recorded. [1]Spatial sampling for Indie shows the point spacing utilised, though this is lower than is normally used for that instrument (normally 0.167 μm). 'Patched' indicates that scans of the same areal extent were where necessary stitched together manually to compensate for lower z range (Indie). '4 median values' indicates where median values of adjacent sub-scans were used in the microscope comparison following the results of the patching analysis.

Nickname	**Institution**	**Make and Model**	**Spatial Sampling (μm)**	**Scan Size (μm^2)**
Connie	University of Arkansas	Sensofar PLμ	0.183	102 x 139 (4 median values)
Indie	University of Indianapolis	Sensofar PLμ 2300	0.664[1]	241 x 181 patched
Bruce	Flinders University	Sensofar PLμ neox	0.166	242 x 181
Wall-E	University of Arkansas	Sensofar PLμ neox	0.166	242 x 181
Dolly	Vanderbilt University	Sensofar PLμ neox	0.166	242 x 181
Zeus	Worcester Polytechnic Institute	Olympus LEXT OLS4000	0.125	243 x 243
Persephone	Worcester Polytechnic Institute	Olympus LEXT OLS4100	0.125	243 x 237

Specimens were compared using the two most common SSFA measures, complexity (Area-scale fractal complexity, *Asfc*) and anisotropy (exact-proportion Length-scale anisotropy of relief, *epLsar*), both of which are standard in DMTA (Scott *et al.* 2006) as well as being used in broader metrological studies (e.g., Pedreschi *et al.* 2000, Quevedo *et al.* 2005, Vessot *et al.* 2015). Complexity (*Asfc*) quantifies how relative area of a scan changes when measured at different scales (Scott *et al.* 2006). The maximum change in relative area found across all scales measured (87 scales between 0.02 and 100 μm), is the returned *Asfc* value. Anisotropy, (*epLsar*) looks at relative length of profiles taken at 5° intervals. The length of each profile in conjunction with its direction creates a vector for each interval, the sum of which is the mean vector length quoted as *epLsar* (Scott *et al.* 2006). All DMTA data were calculated using Sfrax (Surfract, http://surfract.com/products.html).

3.3.3. Analysis

Four analytical phases were undertaken, each considering a different element of DMTA: file type, patching method, microscope, and filter type. The specific experimental design for each phase is featured below, however many elements were universal. Limitations inherent to non-normal distribution of SSFA variables were overcome through transformations. The success of these varied between treatments, as many had vastly different data distributions, including some where strong skew could not be normalized with any transformation (see SI 1). Commonly, complexity data were transformed using a logarithmic transformation, and anisotropy using a square root transform (Crawley 2005, Hammer and Harper 2006). In addition, rank transformations were used for both variables, as is often used in DMTA (e.g., Ungar *et al.* 2008, 2012, Scott *et al.* 2009) and countless other fields of inquiry as a statistically robust solution to non-normally distributed data (Conover and Iman 1981, 1982, Zimmerman and Zumbo 1993). ANOVAs were used to test for differences between microscopes and, where applicable, treatments. Initial tests also included light source (blue

versus white light) as a factor as well as potential interactions with the ‘microscope’ factor, however none showed any significance. Tukey's highly significant differences (HSD) post hoc tests were used to indicate where differences between microscopes lay. All analysis was conducted in R (R Team 2012).

File type

The first comparison sought to test the conversion process required when using Mountains Map (version 7.1.7288, Digital Surf 1996–2014) to modify surfaces. The raw surface file created by the profilers used here (e.g., .plu, .lext extensions) contains both a topography layer and an intensity layer. To apply any filters or modifications to the surface requires that the topography layer alone be extracted, resulting in a .sur surface file. We wanted to test whether this creates any measurable difference to the surfaces. To do this, 1084 .plu scans were compared against the same scans exported as .sur files. This comparison was limited to specimens scanned using Connie, Wall-E, Bruce, and Dolly as those from Zeus and Persephone produced .lext files that could not be read in Sfrax, and those from Indie required sub-scans to be patched in Mountains Map for full comparisons. After initial testing indicated that filling non-measured points on .sur files may alleviate differences between file types, a final set of .sur surfaces with non-measured points filled was compared by an ANOVA comparing 'file type' (with three levels; ‘raw’, ‘export’ and ‘NM filled’. In addition, each export category was compared to each other using both Pearson's and Spearman’s *r* correlation coefficient to further determine whether significantly different treatments still correlate with each other. No suitable mathematical transformations could be found for the file type dataset so rank transformation alone was used for all file type analyses.

Patching

Patching (or stitching when automated) is the digital combination of multiple adjacent scans into one larger scan. The second comparison aimed to determine what effect the size of scans,

or the patching process, may have on the surfaces. This is necessitated as newer microscopes can automatically patch together adjacent scans to attain surfaces of a larger area, while for older microscopes (Connie) this must be done manually or alternatively have their surface quantified by averaging sub-scan data. In addition, patching adjacent scans together requires an overlap between scans to allow sub-scans to be aligned. Because most scans collected previously on Connie have not scanned sufficient overlap to allow full patching, three patching scenarios were used on a dataset of five specimens (*D. lumholtzi* QMJM10086, *M. dorsalis* QMJM13535, *M. rufus* FU 2003.9.14.7, *S. occidentalis* SAMP20981, and *W. bicolor* AMS1924). The first treatment involved patching scans adjacent but with no overlap as a proxy for the existing scans. The second involved allowing for overlap between scans as would be ideal. The third had a 6 μm strip of data removed from the edge of scans to approximate a 'non-ideal' scenario where scans were not entirely contiguous. Measures using the mean and median of sub-scans as is currently practiced were also compared. These methods were compared against the four auto-stitched scans, which were slightly offset from one another in overlapping regions of the same surface to allow for variability between the different methods in terms of their slight differences in area. With the various samples, sub-scans and averaged data used, this resulted in only 70 data points for comparison, which is well below that required for statistical comparison.

Microscope comparison

The third comparison involved comparing 25 specimens of five species (n = 125) across all microscopes. To minimise intra-microscope variability, each specimen was scanned four times and the median of these used in analysis. Sample sizes between microscopes vary somewhat due to technical limitations of some microscopes and difficulty in scanning, but range from n = 5 (Zeus) to n = 52 (Dolly), with a total of 175 individual scans compared for each treatment. Scans taken on Indie often covered comparable areas to other microscopes,

but were limited in depth of field. To overcome this, multiple scans were taken and manually patched as necessary. For Connie, the median values of the four smaller sub-scans were used following inconsistent results of the patching study. Comparisons were made on nine different datasets (table 3.2). Each of these treatments were implemented using Mountains Map operators, and chosen on the basis of visual inspection of scans which showed a number of systematic and random differences between microscopes. The non-measured (NM) filled dataset consisted of .sur files with non-measured points filled, following the results of the file type comparisons. The modified (M) dataset applied a number of operators to the scans, to both assist in editing as well as achieving inter-scope comparability (see Supporting Information 1). Random differences more typical of measurement noise were dealt with by levelling and removing the coarse form of surfaces, before a threshold removed the upper and lower 0.1% of the data. In addition, the automated 'remove outliers' tool in Mountains Map was used to remove any features with a slope > 80°. These two steps removed most 'spikes' characteristic of some measurement noise. To correct for differences in physical parameters of microscopes, scans were resampled in x and y to a spatial sampling of 0.2 μm, which is greater than the sampling interval of most microscopes used, though the point spacing of Indie scans are greater than this. Additionally, all scans were resized to the same size as the smallest scans (220 × 178 μm^2). This region size roughly corresponds to the 276 × 204 μm^2 used by others (e.g., Scott *et al.* 2005, Prideaux *et al.* 2009, DeSantis and Haupt 2014), and was necessitated by the 10% overlap required to enable automated stitching of surfaces on the NEOX profilers. The remaining datasets added filters to remove systematic differences in surface roughness from scans. These filters work by using mathematical functions to identify and remove features which fall below a defined scale. The 2.5 μm cut-off and type of filters used were chosen on the basis of those that minimised intra-specimen variability while maintaining the same shape of the relative area curve. The soft filter (SF) used a single spline

filter; the moderate filter (MF) added a robust gaussian filter to this, and the hard filter (HF) used spline, robust gaussian and gaussian filters. Remaining measurement noise or non-microwear features (e.g., dust) were manually edited out by using the retouch tool in Mountains Map. The effect of this retouching is also investigated here by comparison of edited and unedited surfaces for the M, SF, MF and HF datasets, (with edited datasets abbreviated to modified-edited (M-ed), soft filter-edited (SF-ed), moderate filter-edited (MF-ed) and hard filter-edited (HF-ed) respectively), though not the NM dataset due to the high amount of noise on many scans.

Filter testing

To test what role the various treatments have on the dietary signal, the same filters used in the microscope comparison were applied to the published microwear dataset of Ungar *et al.* (2012) to demonstrate that interspecific differences in microwear remain. The raw .plu and edited .sur files were obtained from the authors, though files for two specimens (*Australopithecus africanus* STS12 and *Homo habilis* STW 15) could not be located. To consider the effect of this, the Ungar *et al.* (2012) data were reanalysed with those two specimens removed. Both the raw .plu and edited .sur files were run through the same filtering process as outlined in table 3.2, with median values of the 4 sub-scans used as per Ungar *et al.* (2012), resulting in 64 values for comparison for each treatment. As the .sur surfaces were edited prior to the Ungar *et al.* (2012) analysis, this allows us to further consider the effect of editing, and in particular look at this at earlier phases than was possible in the microscope comparison phases of analysis.

Table 3.2: Summary of operators used in the microscope comparison study. All were undertaken in the order presented, using Mountains Map Premium 7.1.7288. *Indicates where separate datasets were collected so as to compare the effect of editing scans. See SI 4 for an illustrated example in a Mountains Map document.

Operator	Function	Filled	Modified	Soft Filter	Moderate Filter	Hard Filter
Level	To ensure orientation of specimen does not result in data loss in thresholding. Least squares plane method used.		x	x	x	x
Remove form	To ensure shape of surface does not result in data loss in thresholding. Polynomial 2nd degree method used.		x	x	x	x
Remove outliers	Remove features with slope >80˚ (e.g., measurement noise)		x	x	x	x
Threshold	Remove upper and lower 0.1% of data (e.g., measurement noise)		x	x	x	x
Spline Filter	To remove surface roughness. Cut-off scale 2.5µm					x
Robust Gaussian Filter	To remove surface roughness. Cut-off scale 2.5µm				x	x
Gaussian Filter	To remove surface roughness. Cut-off scale 2.5µm			x	x	x
Retouch	Manually edit features to remove artefacts		*	*	*	*
Fill non-measured points	As suggested following results of file type comparison	x	x	x	x	x
Extract Area	Crop scans to equalise scan region size between microscopes		x	x	x	x
Resample	Resample in x and y to equalise point sampling between microscopes. Spline smoothing used.		x	x	x	x

3.4. Results

File type

The file type ANOVA showed significant differences between file types for both the ranked *Asfc* and ranked *epLsar* data (both $p < 0.001$). Post hoc comparisons for file type were all highly significant for rank *Asfc* (all $p < 0.001$). For rank *epLsar* post hoc comparisons for file type were highly significant when comparing raw and export, and export and filled (both $p <$

0.001), however there was no significant difference between raw and NM filled surfaces (p = 0.110). A series of linear models considered whether, while different, these variables still correlate. These found significant correlations between all pairs for both rank *Asfc* and rank *epLsar* (all $p < 0.001$). Identical results were found for Spearman and Pearson tests. Both variables showed similar trends, with the raw and exported data correlated at $r \sim 0.3$ for ranked *Asfc* and 0.5 ranked *epLsar* (table 3.3). Similarly the raw and NM data correlated at the slightly higher r of ~ 0.5 for ranked *Asfc* and 0.6 for ranked *epLsar*. The exported and NM data showed a higher correlation still, with $r^2 \sim 0.8$, again for both ranked *Asfc* and rank *epLsar.*

Table 3.3: Corelation coefficients for the file type analysis. Lower left is Pearson's r and upper right is Spearman's r. Raw = unmodified .plu files; export = the topology layer extracted and saved as .sur files in Mountains Map; filled = .sur files with non-measured points filled with a smooth algorithm. All *p* values were <0.001. Data collected on the 'Bruce' profiler.

	***Asfc* (complexity)**			***epLsar* (anisotropy)**		
	Raw	**Export**	**Filled**	**Raw**	**Export**	**Filled**
Raw		0.321	0.762		0.506	0.756
Export	0.321		0.545	0.506		0.593
Filled	0.762	0.545		0.756	0.593	

Patching

The sample size for the patching study was too low to allow statistical comparison, but a number of observations can still be made. For complexity, there was substantial variability for autostitched scans, which indicates how sensitive SSFA variables are to slight changes in surface metrology (figure 3.1). Sub-scans showed some variability but were uniformly lower than the autostitched scans, with the mean and median *Asfc* values also being consequently low. Most of the patching techniques similarly failed to achieve consistently comparable *Asfc* measures to the autostitched scans across all specimens, though those patched with overlap

between adjacent scans appear mostly comparable. Similarly, the autostitched scans were highly variable in anisotropy. Sub scan values were similarly variable with the resultant mean and median values of the sub-scans mostly within the range exhibited by the autostitched scans. The patched continuous and patched overlap methods all fell within the range exhibited by the autostitched scans for most specimens, with one exception (*W. bicolor*).

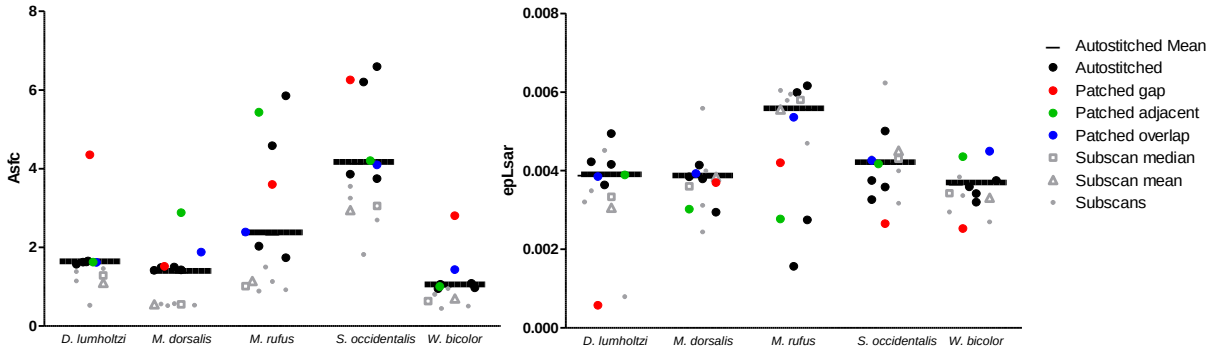


Figure 3.1: Variability in complexity (*Asfc*) and anisotropy (*epLsar*) between patching methods and sub-scan averages. Data collected on the Bruce profiler.

Microscope comparison

Data collected and ANOVA model summaries are presented in SI 2. ANOVA comparisons for species suggest interspecific differences to be present in every dataset (all $p < 0.001$). Inter-microscope differences are reduced in *epLsar* in the modified and modified-edited datasets, but it is not until filters are included that inter-microscope differences are no longer significant for both complexity and anisotropy (table 3.4).

The post hoc comparisons (table 3.5) for the ANOVAs further highlight the differences between microscopes. The mathematically-transformed complexity post hoc comparisons only show significant differences between most microscope comparisons for the 'non-measured filled' dataset. For the modified and modified-edited datasets, a number of significant differences were found in complexity. However, no significant differences were found for any of the filtered datasets (SF, SF-ed, MF, MF-ed, HF, and HF-ed). A similar

Table 3.4: Summary of inter-microscope differences from the microscope comparison ANOVA. Significant differences ($p < 0.05$) in bold. *Asfc* = area-scale fractal complexity; *epLsar*; exact proportion length scale anisotropy; sqrt = square root transform, log = natural logarithm transformation; log10 = common logarithm transformation.

Dataset	Variable	Transformation	p. value
Non-measured filled	Complexity	(*Asfc*)^-0.25	**<0.001**
Non-measured filled	Complexity	rank (*Asfc*)	**<0.001**
Non-measured filled	Anisotropy	no success	N/A
Non-measured filled	Anisotropy	rank (*epLsar*)	**0.001**
Modified	Complexity	log(*Asfc*)	**0.008**
Modified	Complexity	rank (*Asfc*)	**<0.001**
Modified	Anisotropy	sqrt(*epLsar*)	0.063
Modified	Anisotropy	rank (*epLsar*)	**0.032**
Modified-edited	Complexity	log(*Asfc*)	**<0.001**
Modified-edited	Complexity	rank (*Asfc*)	**<0.001**
Modified-edited	Anisotropy	sqrt(*epLsar*)	0.136
Modified-edited	Anisotropy	rank (*epLsar*)	0.146
Soft Filter	Complexity	log(*Asfc*)	0.287
Soft Filter	Complexity	rank (*Asfc*)	0.276
Soft Filter	Anisotropy	none required	0.589
Soft Filter	Anisotropy	rank (*epLsar*)	0.662
Soft Filter-edited	Complexity	log(*Asfc*)	0.698
Soft Filter-edited	Complexity	rank (*Asfc*)	0.687
Soft Filter-edited	Anisotropy	sqrt(*epLsar*)	0.881
Soft Filter-edited	Anisotropy	rank (*epLsar*)	0.919
Moderate Filter	Complexity	log10(*Asfc*)	0.884
Moderate Filter	Complexity	rank (*Asfc*)	0.887
Moderate Filter	Anisotropy	sqrt(*epLsar*)	0.997
Moderate Filter	Anisotropy	rank (*epLsar*)	0.996
Moderate Filter-edited	Complexity	log(*Asfc*)	0.657
Moderate Filter-edited	Complexity	rank (*Asfc*)	0.649
Moderate Filter-edited	Anisotropy	none required	0.940
Moderate Filter-edited	Anisotropy	rank (*epLsar*)	0.953
Hard Filter	Complexity	log10(*Asfc*)	0.832
Hard Filter	Complexity	rank (*Asfc*)	0.811
Hard Filter	Anisotropy	sqrt(*epLsar*)	0.991
Hard Filter	Anisotropy	rank (*epLsar*)	0.938
Hard Filter-edited	Complexity	log(*Asfc*)	0.585
Hard Filter-edited	Complexity	rank (*Asfc*)	0.702
Hard Filter-edited	Anisotropy	none required	0.767
Hard Filter-edited	Anisotropy	rank (*epLsar*)	0.731

story is seen for rank-transformed complexity, with numerous significant post hoc comparisons for the NM, M and M-ed datasets. The filtered datasets again demonstrate no significant differences.

For anisotropy, no suitable transformation was found for the NM filled dataset. Every remaining dataset however, once transformed showed no significant differences between microscopes. In the rank-transformed anisotropy datasets significant differences were only evident in the NM filled and Modified datasets. Four significant post-hoc comparisons were found for the NM filled dataset, and were largely associated with the 'Zeus' profiler (table 3.5). Despite the ANOVA suggesting significant ifferences to be present between profilers in the Modified dataset, the post-hoc comparisons showed no significant differences between any particular pair of microscopes.

Diminished inter-microscope variability is evinced in complexity and anisotropy (figures 3.2–3.3), where all data from each microscope can be compared. Most striking in these are the differences in the NM datasets relative to the remainder. Consistent inter-microscope differences then decrease at each step in the process. In addition, the effect of editing in decreasing variability at each step is seen by comparing edited to non-edited datasets and is most evident in the SF datasets (figures 3.2–3.3). Similarly, improvement in scan variability between datasets is reflected in the standard deviation of the four serial scans taken of each specimen for each microscope (figure 3.4). For complexity this shows the largest standard deviation by far is found in the NM dataset, and that standard deviation is lowered for every subsequent dataset. It also clearly shows standard deviation to be lower on the edited scans compared to the unedited ones. In contrast, the anisotropy standard deviation is fairly uniform across all datasets, with the largest variation being apparent in a small number of outliers associated with the MF and HF datasets.

Table 3.5: Tukey's HSD post hoc comparisons resulting from the microscope comparison ANOVA. Significant differences ($p < 0.05$) in bold. Pers. = Persephone.

	***Asfc* - log or log10 transformed \ rank transformed**							***epLsar* - sqrt transformed (where necessary) \ rank transformed**							
Non-measured filled		Bruce	Connie	Dolly	Indie	Pers.	Wall-E	Zeus		Bruce	Connie	Dolly	Indie	Pers.	Wall-E	Zeus
	Bruce		0.990	0.288	0.975	**<0.001**	**0.002**	**0.020**	Bruce		0.428	0.099	0.953	0.166	**<0.001**	0.827
	Connie	1.000		0.928	0.840	**0.003**	0.072	0.081	Connie			1.000	0.999	0.951	0.270	1.000
	Dolly	0.378	0.847		0.275	**0.013**	0.297	0.223	Dolly				0.995	0.968	0.207	1.000
	Indie	0.384	0.311	**0.018**		**<0.001**	**0.009**	**0.013**	Indie					0.883	0.303	0.998
	Pers.	**<0.001**	**0.001**	**0.005**	**<0.001**		0.576	1.000	Pers.					**<0.001**	**<0.001**	**<0.001**
	Wall-E	**<0.001**	**0.007**	0.066	**<0.001**	0.675		0.902	Wall-E	N/A – no suitable transformation found						0.952
	Zeus	**0.006**	**0.019**	0.083	**<0.001**	1.000	0.869		Zeus							
Modified		Bruce	Connie	Dolly	Indie	Pers.	Wall-E	Zeus		Bruce	Connie	Dolly	Indie	Pers.	Wall-E	Zeus
	Bruce		0.896	0.999	0.872	0.336	0.605	**0.023**	Bruce		0.997	1.000	1.000	0.173	0.681	0.335
	Connie	0.766		0.983	1.000	0.085	0.144	**0.005**	Connie			0.989	0.999	0.105	0.471	0.227
	Dolly	0.990	0.975		0.959	0.188	0.322	**0.011**	Dolly				1.000	0.209	0.762	0.378
	Indie	0.615	0.997	0.864		0.113	0.231	**0.007**	Indie					0.491	0.958	0.555
	Pers.	0.054	**0.005**	**0.014**	**0.006**		0.976	0.782	Pers.						0.865	1.000
	Wall-E	0.903	0.239	0.537	0.210	0.421		0.264	Wall-E	N/A - no significant differences						0.870
	Zeus	**0.001**	**<0.001**	**<0.001**	**<0.001**	0.631	**0.014**		Zeus							
Modified-edited		Bruce	Connie	Dolly	Indie	Pers.	Wall-E	Zeus		Bruce	Connie	Dolly	Indie	Pers.	Wall-E	Zeus
	Bruce		0.646	0.981	0.193	**<0.001**	**0.019**	**0.001**	Bruce							
	Connie	0.488		0.210	0.922	**<0.001**	**<0.001**	**<0.001**	Connie							
	Dolly	0.994	0.168		0.052	**<0.001**	0.109	**0.003**	Dolly							
	Indie	0.073	0.833	**0.021**		**<0.001**	**<0.001**	**<0.001**	Indie	N/A - no significant differences						
	Pers.	**<0.001**	**<0.001**	**<0.001**	**<0.001**		0.142	1.000	Pers.							
	Wall-E	0.064	**0.001**	0.210	**<0.001**	**0.001**		0.207	Wall-E							
	Zeus	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.972	**0.001**		Zeus							

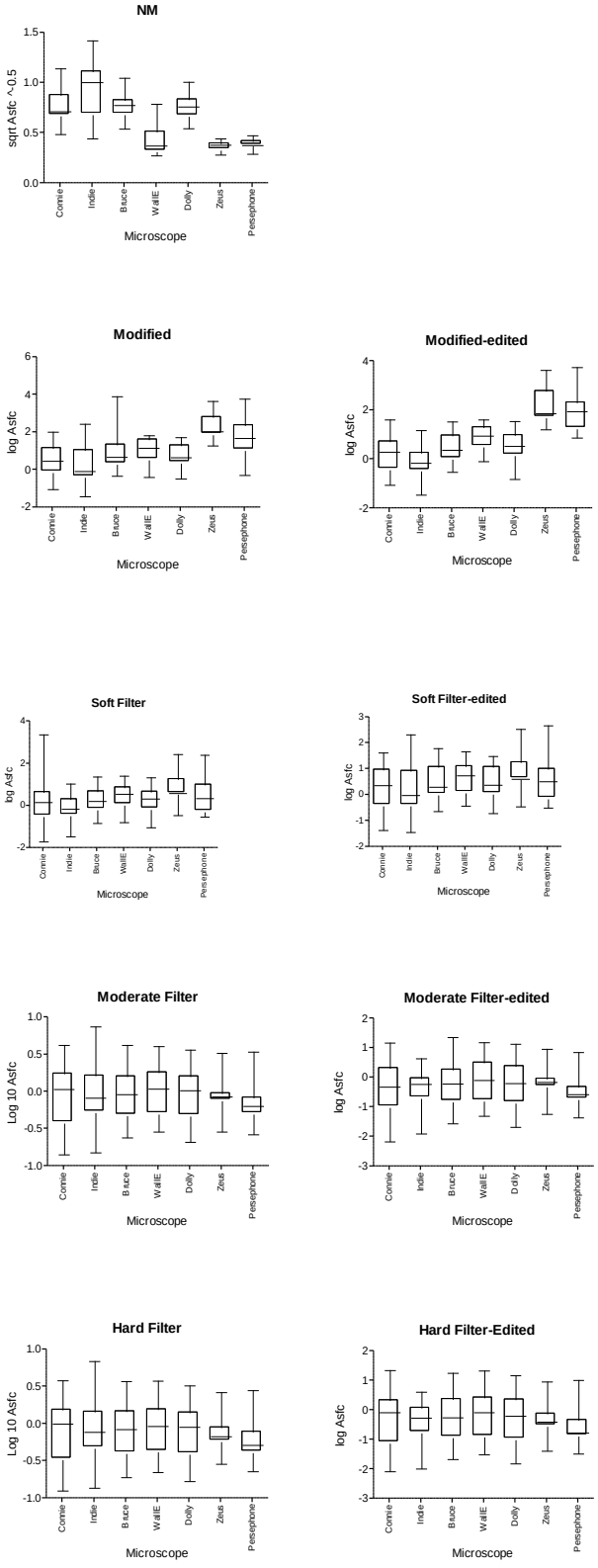


Figure 3.2: Complexity (*Asfc*) boxplots depicting mean (line), 25–75th percentile (box) and range (whiskers) of all scans between microscopes. Note that the transformation used differs between each dataset. Transformations were those required to achieve normality (see SI 1). Data from unedited scans appear left and edited scans right.

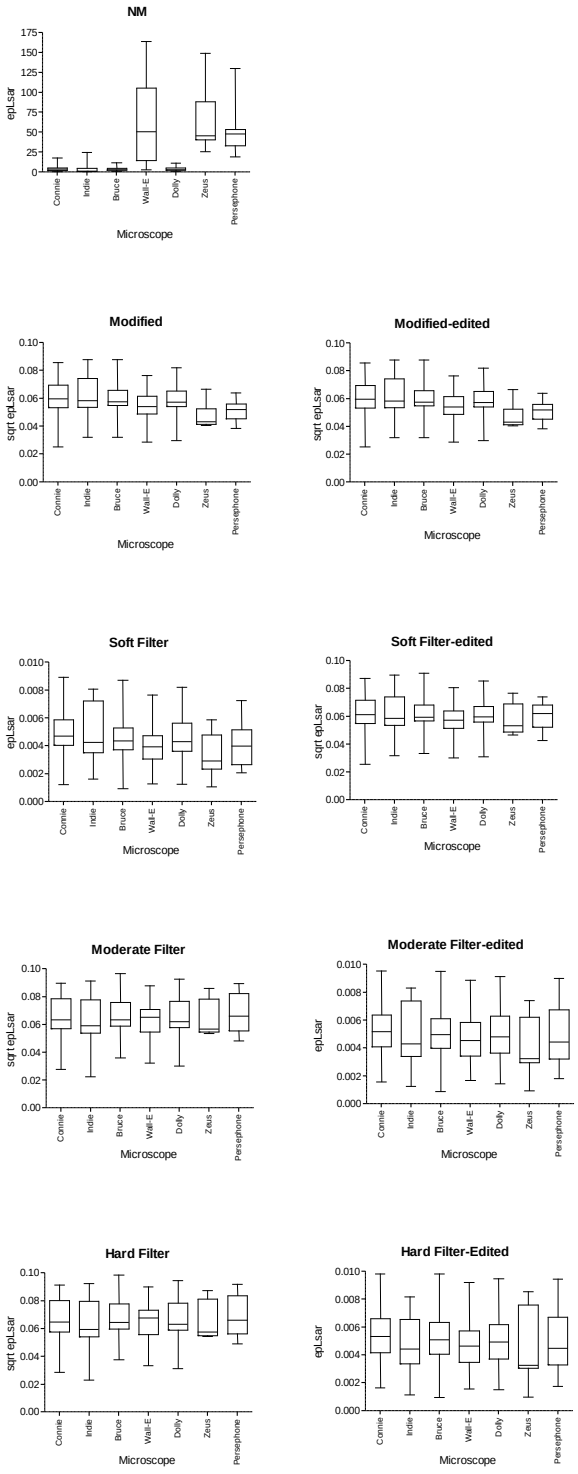


Figure 3.3: Anisotropy (*epLsar*) boxplots depicting mean (line), 25–75th percentile (box) and range (whiskers) of all scans between microscopes. Note that the transformation used differs between each dataset. Transformations were those required to achieve normality (see SI 1), except for NM where no suitable transformation was found so raw *epLsar* values are displayed. Data from unedited scans appear left and edited scans right.

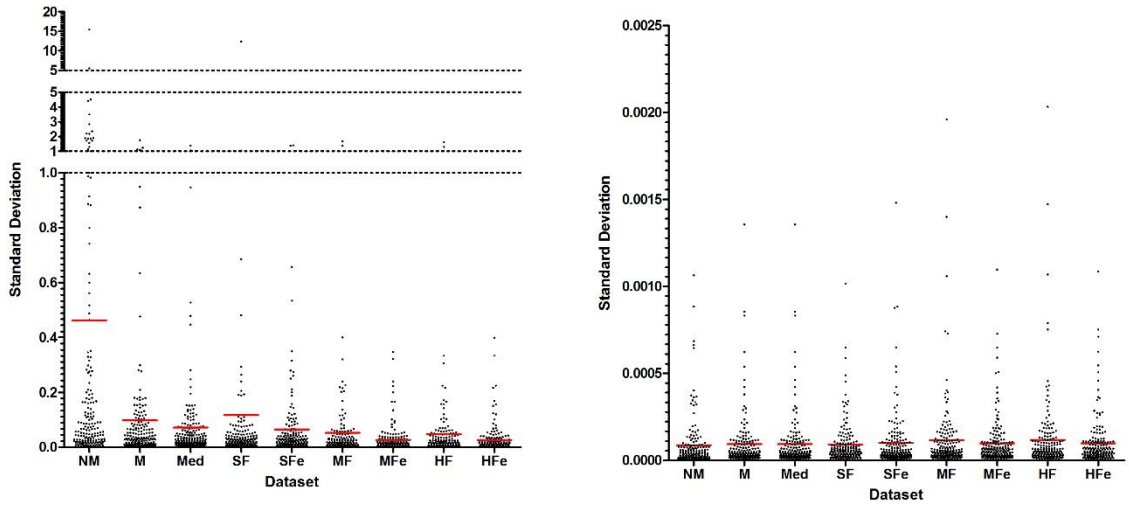


Figure 3.4. Standard deviation of complexity (left) and anisotropy (right). Each point represents the standard deviation of the four scans taken on each specimen at each microscope. Red lines indicates the mean for each dataset. NM = non-measured points filled, M = modified, Med = modified edited, SF = soft filter, SFe = soft filter edited, MF = moderate filter, MFe = moderate filter edited, HF = hard filter, HFe = hard filter edited.

Filter testing

The various processed datasets (SI 3), as well as the reanalysed Ungar *et al.* (2012) dataset all still retain significant differences between species for complexity in both log- and rank-transformed datasets (all $p < 0.05$) (table 3.6). Tukey's HSD post hoc comparisons (table 3.7), show that while the number of significant differences remain consistent, at least for the edited datasets, the specific paired differences change between datasets. One difference was found between the published findings in comparison to those reanalysed here (rank transformed complexity data only). This was the loss of a significant difference between *Australopithecus africanus* and *Homo habilis*, the two species for which scans were missing. Considering the reanalysed Ungar *et al.* (2012) dataset in comparison to the remaining rank complexity datasets, we see the raw .plu data differed by the loss of two interspecific differences

associated with *Australopithecus afarensis*. The raw .sur files however were drastically different, with only two interspecific differences remaining. The edited .sur dataset, NM-edited and M-ed datasets however were much more comparable to the Ungar *et al.* (2012) dataset. Considering the remaining data for rank complexity, only two interspecific differences were maintained through every dataset (*A. afarensis* versus *Paranthropus robustus*, and *P. robustus* versus *P. boisei*). The exact interspecific differences were somewhat variable, though some trends were evident. For every treatment, the unedited surfaces showed less significantly different post hoc comparisons than the edited surfaces. Of the filtered datasets where microscope differences were shown to be minimised, the SF-ed data appeared most comparable, with only two differences found in interspecific pairings between the SF-ed and reanalysed Ungar *et al.* (2012) post hoc comparisons.

For the log-complexity data, comparisons were limited by suitable transformations not being found for some datasets. Again the unedited .sur dataset was vastly different to the remainder, with only one significant interspecific difference found. The reanalysed Ungar *et al.* (2012) dataset, .sur edited and NM filled-edited datasets were near identical in post hoc comparisons. The raw, M-ed and SF datasets did not produce any interspecific comparisons that were not seen in the reanalysed data of Ungar *et al.* (2012), but overall showed less significant differences. The MF-ed and HF-ed datasets produced four differences in interspecific significant differences to the reanalysed data of Ungar *et al.* (2012). Edited datasets in general showed more interspecific differences, which subsequently resulted in the MF and HF not showing any contradictions to Ungar *et al.* (2012). The post hoc differences can be seen in the log complexity boxplots (figure 3.5). For each dataset in figure 3.5 the broad trends of the original Ungar *et al.* (2012) dataset are still apparent, however as more processes are applied, interspecific differences become minimised. The editing process also appears to have reduced the range of many taxa.

Comparisons of *epLsar* between datasets are more similar to results in Ungar *et al.* (2012), which found no significant differences between species, as was found for most datasets here with a small number of exceptions (table 3.6). The post hocs for these datasets are less than illuminating, with the only significant interspecific pair for rank NM being *Australopithecus africanus* versus *Paranthropus robustus*, ($p = 0.022$) while for square-root raw (.sur), rank raw (.sur), square-root M, rank M and rank M-ed, no significantly different ($p < 0.05$) post hoc pairs were identified.

Table 3.6: Summary of interspecific differences from the filter testing ANOVA. Significant differences ($p < 0.05$) in bold. 'Published results' are those of Ungar *et al.* (2012); *Asfc* = area-scale fractal complexity; *epLsar*; exact proportion length scale anisotropy; sqrt = square root transform, log = natural logarithm transformation. 'None found' in transformation indicates that no transformations could be found which resulted in a normal distribution. 'None required' indicates that the raw data were normally distributed.

Dataset	Variable	Transformation	p. value
Published results	Complexity	rank (*Asfc*)	**<0.001**
Published results	Anisotropy	rank (*epLsar*)	0.118
Re-analysis of published	Complexity	log(*Asfc*)	**<0.001**
Re-analysis of published	Complexity	rank (*Asfc*)	**<0.001**
Re-analysis of published	Anisotropy	sqrt(*epLsar*)	0.078
Re-analysis of published	Anisotropy	rank (*epLsar*)	0.066
Raw (.plu)	Complexity	log(*Asfc*)	**<0.001**
Raw (.plu)	Complexity	rank (*Asfc*)	**<0.001**
Raw (.plu)	Anisotropy	None required	0.769
Raw (.plu)	Anisotropy	rank (*epLsar*)	0.776
Raw (.sur)	Complexity	log(*Asfc*)	**0.007**
Raw (.sur)	Complexity	rank (*Asfc*)	**0.007**
Raw (.sur)	Anisotropy	sqrt(*epLsar*)	**0.042**
Raw (.sur)	Anisotropy	rank (*epLsar*)	**0.035**
Edited (.sur)	Complexity	log(*Asfc*)	**<0.001**
Edited (.sur)	Complexity	rank (*Asfc*)	**<0.001**
Edited (.sur)	Anisotropy	sqrt(*epLsar*)	0.062
Edited (.sur)	Anisotropy	rank (*epLsar*)	0.061
Non-measured filled	Complexity	None found	N/A
Non-measured filled	Complexity	rank (*Asfc*)	**<0.001**
Non-measured filled	Anisotropy	None required	0.742
Non-measured filled	Anisotropy	rank (*epLsar*)	0.735
Non-measured filled-edited	Complexity	log(*Asfc*)	**<0.001**
Non-measured filled-edited	Complexity	rank (*Asfc*)	**<0.001**
Non-measured filled-edited	Anisotropy	sqrt(*epLsar*)	0.061
Non-measured filled-edited	Anisotropy	rank (*epLsar*)	**0.043**
Modified	Complexity	None found	N/A
Modified	Complexity	rank (*Asfc*)	**<0.001**
Modified	Anisotropy	sqrt(*epLsar*)	**0.050**
Modified	Anisotropy	rank (*epLsar*)	**0.046**
Modified-edited	Complexity	log(*Asfc*)	**<0.001**
Modified-edited	Complexity	rank (*Asfc*)	**<0.001**
Modified-edited	Anisotropy	sqrt(*epLsar*)	0.053
Modified-edited	Anisotropy	rank (*epLsar*)	**0.049**

Table 3.6 (continued).

Dataset	Variable	Transformation	p. value
Soft Filter	Complexity	log(*Asfc*)	**<0.001**
Soft Filter	Complexity	rank (*Asfc*)	**<0.001**
Soft Filter	Anisotropy	sqrt(*epLsar*)	0.127
Soft Filter	Anisotropy	rank (*epLsar*)	0.138
Soft Filter-edited	Complexity	log(*Asfc*)	**<0.001**
Soft Filter-edited	Complexity	rank (*Asfc*)	**<0.001**
Soft Filter-edited	Anisotropy	sqrt(*epLsar*)	0.162
Soft Filter-edited	Anisotropy	rank (*epLsar*)	0.107
Moderate Filter	Complexity	log(*Asfc*)	**<0.001**
Moderate Filter	Complexity	rank (*Asfc*)	**<0.001**
Moderate Filter	Anisotropy	sqrt(*epLsar*)	0.167
Moderate Filter	Anisotropy	rank (*epLsar*)	0.101
Moderate Filter-edited	Complexity	log(*Asfc*)	**<0.001**
Moderate Filter-edited	Complexity	rank (*Asfc*)	**<0.001**
Moderate Filter-edited	Anisotropy	sqrt(*epLsar*)	0.184
Moderate Filter-edited	Anisotropy	rank (*epLsar*)	0.126
Hard Filter	Complexity	log(*Asfc*)	**<0.001**
Hard Filter	Complexity	rank (*Asfc*)	**<0.001**
Hard Filter	Anisotropy	sqrt(*epLsar*)	0.189
Hard Filter	Anisotropy	rank (*epLsar*)	0.130
Hard Filter-edited	Complexity	log(*Asfc*)	**<0.001**
Hard Filter-edited	Complexity	rank (*Asfc*)	**<0.001**
Hard Filter-edited	Anisotropy	sqrt(*epLsar*)	0.204
Hard Filter-edited	Anisotropy	rank (*epLsar*)	0.154

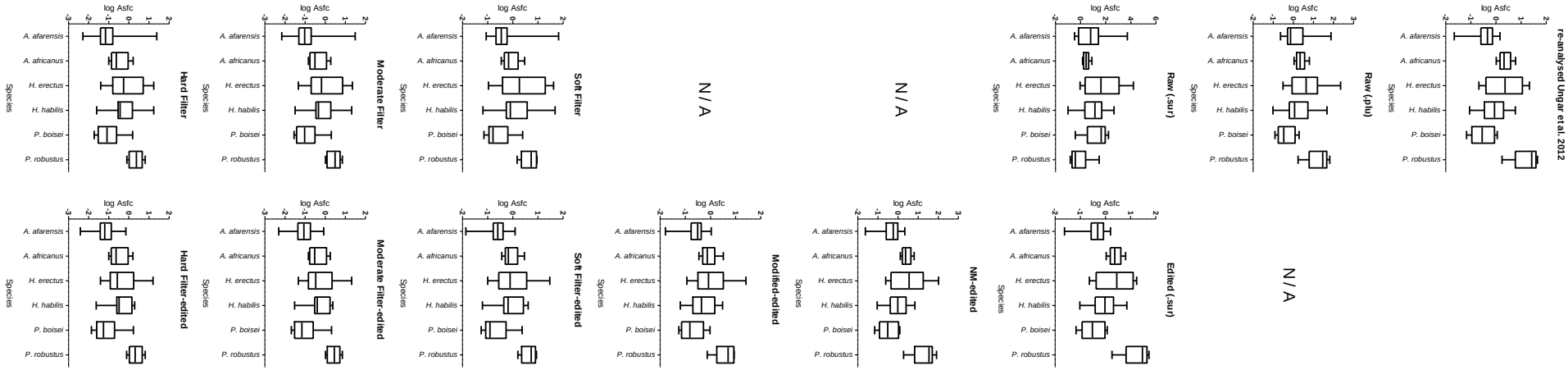
Table 3.7 (pages 99–101): Tukey's HSD post hoc interspecific comparisons resulting from the filter testing ANOVA. Significant differences ($p < 0.05$) in bold. 2012 published results and data refer to Ungar *et al.* (2012). For the 2012 published results 'B' refers to significance in both Tukey's HSD and Fisher's LSD post hoc comparisons, 'C' indicates significance in Fisher's LSD alone, and '0' indicates significance in neither.

	***Asfc* - log transformed \ rank transformed**					
2012 Published Results		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		**B**	**B**	0	0	**B**
	A. africanus			0	**B**	**B**	C
	H. erectus				0	**B**	**B**
	H. habilis					C	**B**
	P. boisei						**B**
	P. robustus						
2012 data re-analysed		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		**0.000**	**0.023**	0.638	0.842	**0.000**
	A. africanus	**0.005**		0.909	0.107	**0.000**	0.323
	H. erectus	**0.014**	1.000		0.649	**0.005**	**0.042**
	H. habilis	0.746	0.333	0.456		0.194	**0.000**
	P. boisei	0.913	**0.002**	**0.004**	0.334		**0.000**
	P. robustus	**0.000**	**0.009**	**0.008**	**0.000**	**0.000**	
Raw (.plu)		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		0.468	0.254	0.989	0.308	**0.000**
	A. africanus	0.889		0.999	0.908	**0.017**	0.190
	H. erectus	0.232	0.897		0.730	**0.007**	0.423
	H. habilis	0.997	0.995	0.625		0.203	**0.016**
	P. boisei	0.367	0.108	**0.008**	0.303		**0.000**
	P. robustus	**0.000**	**0.040**	0.419	**0.009**	**0.000**	
Raw (.sur)		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		0.947	0.516	0.986	0.719	0.155
	A. africanus	0.933		0.233	0.768	0.367	0.760
	H. erectus	0.210	0.074		0.929	0.999	**0.010**
	H. habilis	0.999	0.874	0.534		0.987	0.097
	P. boisei	0.906	0.540	0.862	0.992		**0.019**
	P. robustus	0.234	0.874	**0.004**	0.241	0.070	
Edited (.sur)		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		**0.000**	**0.020**	0.690	0.819	**0.000**
	A. africanus	**0.006**		0.950	0.115	**0.000**	0.309
	H. erectus	**0.015**	1.000		0.578	**0.004**	0.056
	H. habilis	0.745	0.355	0.471		0.206	**0.000**
	P. boisei	0.904	**0.002**	**0.004**	0.320		**0.000**
	P. robustus	**0.000**	**0.008**	**0.007**	**0.000**	**0.000**	
Non-measured filled		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		0.718	0.146	0.986	0.186	**0.002**
	A. africanus			0.924	0.986	**0.023**	0.196
	H. erectus				0.596	**0.002**	0.788
	H. habilis					0.116	**0.045**
	P. boisei						**0.000**
	P. robustus						

	***Asfc* - log transformed \ rank transformed**					
Non-measured filled - edit		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		**0.001**	**0.015**	0.693	0.809	**0.000**
	A. africanus	**0.021**		0.988	0.161	**0.000**	0.359
	H. erectus	**0.007**	0.998		0.517	**0.003**	0.123
	H. habilis	0.820	0.499	0.273		0.201	**0.001**
	P. boisei	0.909	**0.006**	**0.002**	0.393		**0.000**
	P. robustus	**0.000**	**0.012**	0.052	**0.000**	**0.000**	
Modified		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		0.454	0.156	0.857	0.593	**0.000**
	A. africanus			0.991	0.992	**0.049**	0.194
	H. erectus				0.851	**0.012**	0.555
	H. habilis					0.182	0.053
	P. boisei						**0.000**
	P. robustus						
Modified - edit		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		**0.018**	0.072	0.417	0.973	**0.000**
	A. africanus	0.083		0.999	0.819	**0.011**	0.241
	H. erectus	**0.032**	0.998		0.956	**0.037**	0.140
	H. habilis	0.633	0.910	0.719		0.219	**0.013**
	P. boisei	0.957	**0.036**	**0.014**	0.323		**0.000**
	P. robustus	**0.000**	0.050	0.162	**0.003**	**0.000**	
Soft Filter		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		0.394	0.068	0.143	0.920	**0.000**
	A. africanus	0.844		0.958	0.997	0.140	0.085
	H. erectus	0.084	0.733		0.999	**0.021**	0.469
	H. habilis	0.408	0.989	0.969		**0.045**	0.233
	P. boisei	0.910	0.420	**0.024**	0.138		**0.000**
	P. robustus	**0.001**	0.125	0.883	0.394	**0.001**	
Soft Filter - Edit		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		**0.018**	**0.046**	**0.037**	0.996	**0.000**
	A. africanus	0.070		1.000	1.000	**0.021**	0.083
	H. erectus	**0.030**	0.999		1.000	**0.044**	0.065
	H. habilis	0.096	1.000	0.996		**0.038**	**0.048**
	P. boisei	0.998	0.078	**0.036**	0.102		**0.000**
	P. robustus	**0.000**	**0.035**	0.113	**0.026**	**0.000**	

	***Asfc* - log transformed \ rank transformed**					
Moderate Filter		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		0.225	**0.022**	0.053	1.000	**0.000**
	A. africanus	0.454		0.936	0.993	0.301	0.081
	H. erectus	**0.035**	0.858		0.999	**0.047**	0.517
	H. habilis	0.104	0.982	0.997		0.098	0.262
	P. boisei	1.000	0.527	0.070	0.170		**0.000**
	P. robustus	**0.000**	0.106	0.725	0.392	**0.001**	
Moderate Filter - Edit		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		**0.028**	**0.024**	**0.006**	1.000	**0.000**
	A. africanus	**0.049**		1.000	0.997	0.073	**0.041**
	H. erectus	**0.017**	0.998		1.000	0.060	0.077
	H. habilis	**0.018**	1.000	1.000		**0.023**	0.123
	P. boisei	1.000	0.163	0.071	0.081		**0.000**
	P. robustus	**0.000**	**0.033**	0.122	0.074	**0.000**	
Hard Filter		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		0.219	**0.019**	**0.049**	1.000	**0.000**
	A. africanus	0.412		0.925	0.992	0.291	0.083
	H. erectus	**0.032**	0.870		0.998	**0.040**	0.548
	H. habilis	0.086	0.980	0.998		0.090	0.275
	P. boisei	1.000	0.520	0.073	0.162		**0.000**
	P. robustus	**0.000**	0.104	0.702	0.393	**0.001**	
Hard Filter - edit		*A. afarensis*	*A. africanus*	*H. erectus*	*H. habilis*	*P. boisei*	*P. robustus*
	A. afarensis		**0.029**	**0.016**	**0.004**	1.000	**0.000**
	A. africanus	**0.044**		1.000	0.993	0.081	**0.039**
	H. erectus	**0.015**	0.997		1.000	**0.047**	0.102
	H. habilis	**0.014**	0.999	1.000		**0.019**	0.146
	P. boisei	1.000	0.168	0.072	0.077		**0.000**
	P. robustus	**0.000**	**0.036**	0.134	0.087	**0.000**	

Figure 3.5: Log complexity (*Asfc*) boxplots depicting mean (line), 25–75th percentile (box) and range (whiskers) of the filter testing datasets. Note that although all have been figured using log transformations, the NM filled and moderate datasets did not achieve normality through this transformation and are figured for comparative purposes only. Data from unedited scans appear left and edited scans right.



3.5. Discussion

The numerous datasets presented here have a number of implications for DMTA research protocols. Data for DMTA variables collected on seemingly identical scans are altered considerably by various factors including file type, patching method and, most importantly, profiler. The use of semi-automated operators, however, provides a simple methodology to negate much of this variability, though the effect of this on the dietary signal is unclear. To keep the discussion in context, each analytical phase is discussed in turn before considering how the results can be implemented.

File type

The file type comparisons show that the simple transformation of one file type into another significantly alters the data. This was reinforced by the filter testing analysis where results for raw .sur surfaces differed markedly from those on both raw .plu surfaces and NM filled surfaces. However, this may in part reflect of how Sfrax reads the file types, and understanding this may be the key to working out the most appropriate methodology. The greater correlation between the raw .plu and filled .sur surfaces in the linear models and filter testing suggests that filling the non-measured points on .sur files preserves the greatest amount of information contained in the .plu files. This is further illustrated by the lack of significant differences between these two treatments alone found in the file type rank *epLsar* ANOVA.

Patching

None of the patching techniques or averages of sub-scans appear to correlate reliably with the autostitched scans. The most reliable technique for approximating autostitched scans seems the 'optimal' conditions of patched with overlap, which is unavailable for the vast majority of DMTA data. Sub-scan mean or median values are mostly comparable for anisotropy but consistently lower for complexity. This fits with our current knowledge of DMTA, where

changes in complexity under subdivisions of scans is used as a variable itself: heterogeneity of complexity (Scott *et al.* 2006). The consistently higher complexity of the autostitched scans suggests that larger scans may be better at quantifying higher complexity values. It may even be that larger scans still will continue this trend and allow greater investigation of complexity. Ultimately, a more thorough study of intra-facet variability is needed to determine what scan size is optimal. Despite differences, however, the microscope comparison showed that the median values for sub-scans taken on Connie were unable to be differentiated from the autostitched scans of other microscopes in a number of datasets. Indeed, comparing complexity between microscopes (figure 3.2), *Asfc* sub-scan median values from Connie were in fact lower than most for the M and M-ed datasets as would be expected, but not for the filtered datasets. This suggests that while complexity may be lower when using sub-scan medians, this issue is either outweighed by other factors, or alleviated by the filtering process.

Microscope comparison

The findings of the microscope comparison are quite clear. For the NM Filled, modified (M), and modified edited (M-ed) datasets, differences between microscopes are too significant to allow data collected from different microscopes to be considered together. Differences between microscopes in the NM dataset are unsurprising, given that the point spacing and hence scale at which complexity and anisotropy are measured differ between microscopes. This allows lower point spacing microscopes to quantify data that are literally off the scale of lower point-spacing microscopes. That significant differences exist in the M and M-ed datasets show that it is likely measurement noise between microscopes rather than just point spacing that is determining differences. All filtered datasets (SF, SF-ed, MF, MF-ed, HF and HF-ed) showed no differences between microscopes and so would appear to be the best choice for minimising microscope variability.

Filter testing

Differences between the original results of Ungar *et al.* (2012) and the reanalysis of the same dataset, minus the two missing specimens is an interesting aside as it shows how crucial sample sizes can be for demonstrating interspecific differences. Similarities in interspecific differences between the dataset of Ungar *et al.* (2012) and the NM-ed dataset are also reassuring because, as seen in the file type analysis, there was some concern for how non-measured points were being interpreted by the algorithms. This is particularly true because Ungar *et al.* (2012) used the software Toothfrax to quantify the SSFA variables, while this study used Sfrax. Moreover, the file type analysis as well as the data used in this analysis (SI 3), show that the *Asfc* values produced do differ. These results demonstrate that, while the numbers themselves differ due to filling of NM points and software specifications, the dietary signal remains.

The relative effectiveness of the various datasets in preserving dietary differences is difficult to clearly establish, because little difference was evident between many datasets in terms of number of significant post hoc pairs. Instead, many datasets, especially MF and HF, differed in which actual pairs were differentiated. As the aim, however, was to replicate the published results, the failure of the filtered datasets to fully replicate the original findings indicates that these standards have not been met. That said, one possibility to consider is that the filters used here were intended to remove inter-microscope variability, much of which is measurement noise. If the filters are indeed removing this noise, the altered dietary differences noted may in fact be a more 'pure' dietary signal, with the raw dietary signal in fact being obscured or altered by measurement noise. It should be noted however, that despite this noise, single-microscope studies have had success in differentiating dietary groups. This shows that even if measurement noise is a factor, the dietary signal has been sufficiently strong to rise above it. The extent of noise in comparison to dietary signal can only be

evaluated through the application of these filters to other datasets, particularly those of extant species whose diets are known or by in vitro and in vivo studies where the diet variables are controlled to a greater extent than possible using museum collections.

It may be tempting to compare the inter-microscope effects noted here to the inter-observer error in SEM based microwear (Grine *et al.* 2002, Galbany *et al.* 2005) or even binocular-light microscopy studies (Mihlbachler *et al.* 2012). The effects noted by these researchers, however, relate to the observer measurement, rather than scanning itself. In this way these issues are not entirely comparable, and it may be expected that similar intra- and inter-observer effects would exist if different researchers were to manually quantify a confocal photosimulation. Similar inter-SEM effects are also almost certainly present, and differences in SEM images are known to be related to the instrument settings (Gordon 1988, Ungar *et al.* 2003). Only the DTMA approach, through algorithmic quantification of surfaces allows observer-measurement independent comparison to be undertaken, and particularly allows these effects to be continually monitored as data is accumulated over time.

Implementing the findings

The microscope comparison study demonstrates that it was not until the inclusion of the filters that microscope comparability could be achieved. In contrast, the filter testing section demonstrated that substantial differences to the original findings of Ungar et al. (2012) become apparent when more filters are used. With these two factors seemingly running counter to one another, the most reasonable suggestion may be the use of the SF-ed treatment. This method was able to negate differences between microscopes while maintaining most of the interspecific differences of Ungar *et al.* (2012). More work needs to be done, however, to find the best balance between minimising inter-microscope differences without compromising the dietary signal. The exact specifications of the filters used here were chosen largely by subjective comparisons, and a more systematic analysis of similar

filters may produce a better result. Ultimately, the utility of this will be determined by applying the filter to a number of datasets collected on different instruments, and including microscope as a factor in analysis.

The suggestions here that the wealth of data collected to date are not comparable between microscopes may appear alarming to some, but it is a solvable problem. The filtering process applied here can be implemented, even for very large datasets by using the 'Template' function in Mountains Map, using the provided workflow (SI 4) as a guide. Moving forward, filtered scans, raw .plu and edited .sur files suitable for inter-microscope dietary analysis could also be uploaded onto any of the increasing number of online databases (e.g., Atlas of Prehistoric Australia, RUcore, Dryad). This would allow others to easily compile large inter-microscope, and more importantly interspecies datasets for comparison. By boosting sample size this approach would also add veracity to statistical comparisons, and extend the scope of conclusions that can be made. It would also ameliorate the problems encountered in the filter testing stage here where two scans from Ungar *et al.* (2012) could not be located.

Some features of DMTA processes that have been investigated here should be noted. Differences between edited and unedited surfaces throughout the filter testing highlight the need to edit surfaces to maintain interspecific differences, though whether inter-operator errors could be encountered in editing needs testing.

The x–y resampling on the M and subsequent datasets resampled at below the resolution of most microscopes, except Indie, where surfaces were scanned at > 3× coarser resolution than the resampled scans. Despite this, Indie scans were not uniformly different from other microscopes in most analyses. The implications of this in terms of how resampling relates to scan resolution is unclear, but the considerable difference between the M and NM datasets do

suggest that the resampling and/or the other treatments applied in the M dataset still minimise some inter-microscope differences.

In the patching study, variability was noted in the autostitched scans for some specimens between slightly offset scans. This may be a result of slight changes in surface topology as the scan was laterally shifted. That the DMTA variables can be so dramatically altered by small shifts across the surface (all scans still covered > 95% of the same surface) suggest that DMTA variables are highly sensitive. This may also explain some variability between microscopes, as the precise orientation of the specimen scanned is difficult to control.

Considering the standard deviation plot (figure 3.4), it becomes clear that even with the most marked filters and editing, there is still considerable variation even between serial scans of the same specimen. This shows that even under the best of conditions, there is some inherent noise in dental microwear texture characterization. This noise is of fundamental importance in considering what constitutes dietary differences, especially when sample sizes are low. In some cases, instrument noise may obscure subtle but meaningful variation in microwear patterning and so define the limits of DMTA to resolve such differences.

This study also attempted a number of patching methods with varying success. As none of the patching methods were entirely successful, and considerable time would be required to patch together the wealth of data collected on Connie, it seems that there would be little to gain from patching existing data. While it is less than perfect, the sub-scan median seems to be the most applicable technique, and is consistent with current practice.

Mathematical transformations have traditionally been shunned by DMTA researchers, with most preferring to use rank transformations. Rank transformations are often chosen as outliers in data make it difficult to achieve normality through mathematical transformations, as well as being more statistically conservative. Indeed finding suitable transformations

limited some analysis here. However, lack of suitable transformations were only encountered when dealing with raw, NM filled or modified datasets. For all M-ed datasets or any dataset where filters were used, normality was achieved through mathematical transformations. This may suggest that the processes undertaken here to minimise inter-microscope differences also help bring outlying data into more consistent trends and in doing so assist in attaining normal distributions of data. Whether this will assist in differentiating dietary differences beyond what is seen with rank transformations is unclear at this stage, and again requires further testing with larger datasets. At this stage, however, there is no indication of consistent differences between rank- and mathematically-transformed data.

This study has focused on complexity and anisotropy, arguably the two most commonly used SSFA DMTA variables. Whether the results here would apply equally to other variables remains to be seen. Some SSFA variables, such as scale of maximum complexity and heterogeneity of complexity are directly related to complexity and so are expected to behave in similar ways to that measure. Other scale-sensitive measures, such as textural fill volume, however, may be more sensitive to finer-scale differences between surfaces so will need to be similarly tested. STA measures too will need to be similarly compared between microscopes. Indeed, as many STA parameters are related to absolute rather than scale-sensitive measures, these variables are likely to be more affected by differences in microscope resolution, and so will need to be similarly compared between microscopes.

The process used in this study demonstrably altered DMTA variables. The file type and filter testing analyses showed that even converting formats or using different software to calculate the DMTA data alter the raw signal. This suggests that an acknowledgement should be made that we are no longer dealing with DMTA values inherent to a specimen, and consider instead that the output relies on a surface–microscope–metrology–analysis interaction. Indeed, the contrast between the output DMTA variables and their analysed outcome is often ignored

with researchers preferring to discuss and figure raw data rather than the transformed data on which the analysis was undertaken (e.g., Ungar *et al.* 2012, DeSantis and Haupt 2014, Shearer *et al.* 2015). This is likely done to allow comparison between studies, but as seen here the numerous elements involved in DMTA data collection differ markedly, so comparison can only really be allowed when all elements are identical between studies. Moreover as the rank transformation most commonly utilised is inherently tied to the dataset in question, true replication is virtually impossible. Instead, to move forward, researchers must not compare data between studies directly, particularly when different instruments or protocols using those instruments are employed. By including data collected elsewhere, adequately filtered, and considering methodological differences as factors, DMTA can continue to build upon the dental microwear record.

3.6. Conclusions

This study shows that the same surface scanned on different microscopes can produce substantially different DMTA data. Indeed, variability exists between scans slightly offset laterally, or even serially scanned under identical parameters. Much of this variability can plausibly be attributed to instrument measurement noise, compounded by sensitivity of SSFA-based DMTA itself. The operators presented here (SI 4) reduce variability between microscopes and enable data collected from instruments of varied specifications to be reliably compared.

Our findings were only made possible through the systematic manipulation and analysis of surfaces through DMTA, which are unavailable for SEM or light microscopy methods. Numerous other aspects of DMTA data collection could benefit from a similar approach. In particular such a systematic approach could help alleviate inter-scan variability within an instrument by fine tuning factors in data collection such as light intensity or type, size of scan, or method used in converting point clouds to surfaces. There are also many aspects

presented here that need further testing through implementation. The work presented here, along with that of others (e.g., Scott *et al.* 2006, Goodall *et al.* 2015), represents an ongoing effort to refine and improve DMTA methods to maximize the ability of researchers to elucidate information on palaeodiet as well in other metrological investigations.

3.7. Acknowledgments

Financial support was provided by a Flinders University Research Scholarship and Flinders University Overseas Field Trip Grant to SDA and an Australian Research Council grant (DP110100726) to GJP. This work would not have been possible without funding of the named profilers here discussed, this includes Australian Research Council grant LE13010115 to GJP, and a National Science Foundation (USA) grant to LD (NSF EAR 1053839). Thanks to Rob Scott for providing the scans used in the Filter Testing section. Thanks to Dom, Keith, Chris, Jeremy and anyone else who let SDA crash on their couch in the US.

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Chapter 4

Incorporating intraspecific variability into dental microwear texture analysis

'*There is poetry in science, but also a lot of bookkeeping.'*

-Sir Peter Medawar, 1996*

4.0. Context

This chapter incorporates within-species variation into analyses of DMTA data. Such sources of variation cover data collection, such as those documented in chapter three, as well as biological variation, such as geographical origin of specimens. Here we show that through modelling we can incorporate such variation into analyses, improving our ability to differentiate diets, and at the same time increasing the available sample sizes for analysis.

4.0.1. Statement of Authorship

Sam Arman designed the study, collected and analysed the data, and wrote the manuscript.

Thomas Prowse wrote the GLMM script and helped draft the manuscript.

Aiden Couzens wrote the subsampling script and helped draft the manuscript.

Peter Ungar helped draft the manuscript.

Gavin Prideaux conceived the study and helped draft the manuscript.

* Medawar, P. B. (1996). *The Strange Case of the Spotted Mice and Other Classic Essays on Science*. Oxford University Press.

Incorporating Intraspecific Variability into Dental Microwear Texture Analysis

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4.1. Abstract

Dental microwear texture analysis (DMTA) is a powerful tool for investigating diets of extinct animals. Most analyses to date have focused on differentiating diet at a species or dietary-group level, typically by controlling for, rather than incorporating intraspecific variability. This limits the sample sizes available for many fossil taxa, because inherently-limited palaeontological sample sizes are further lowered by limiting sampling to specific teeth/facets etc., curtailing the potential scope of conclusions. Here we investigate intraspecific variability in macropodid (kangaroo) microwear to understand how data vary and to allow for broader sampling protocols. Within species, we demonstrate significant variability for every factor considered here for both scale sensitive fractal analysis (SSFA), as well as standard surface texture analysis (STA) variables. Intraspecific factors were then incorporated into interspecific (dietary) analyses through the use of general linear mixed modelling (GLMM), incorporating Akaike's Information Criterion (AIC) to compare models

and testing models developed through independent cross-validation. This revealed that for each DMTA variable only a small number of intraspecific factors need to be included to best delineate between the species sampled. In particular, including *specimen* as a random factor accounts for natural inter-individual variation. A number of other factors featured in final models determined by GLMM, particularly *facet*, *ecoregion* and *microscope*, and increase precision in differentiating dietary groups. *Ecoregions*, a global spatial dataset that defines regions based on climatological and biological parameters, also may potentially allow coarse habitat to be inferred for extinct taxa. We conclude that models of DMTA data that include intraspecific variability allow for broader sampling, and improve the resolution with which dental microwear analyses can be used to infer past diet.

4.2. Introduction

Dental microwear is the collective term for fine-scale surface features (e.g., pits, scratches) left on teeth from physico-chemical interactions. Methods to study microwear have varied considerably over time (DeSantis *et al.* 2013), culminating to date in dental microwear texture analysis (DMTA), which applies algorithmic quantification, using scale-sensitive fractal analysis (SSFA) (Scott *et al.* 2006), or ISO standard surface texture analysis (STA) (Calandra and Merceron 2016), to 3D surfaces scanned by confocal profilers (Ungar *et al.* 2003).

When scanning surfaces for microwear, researchers often limit scanning to specific wear facets and/or teeth to assure comparability of results across samples (e.g., Scott *et al.* 2006, Stynder *et al.* 2012, Merceron *et al.* 2014), following earlier feature-based work (e.g., Gordon 1982, 1984, Teaford and Walker 1984). As DMTA is focussed on dietary inference for palaeontological taxa however, such sampling practices quickly deplete available specimens. Indeed, some authors have resorted to sampling different teeth to increase sample sizes for comparison (e.g., Ungar *et al.* 2012, Souron *et al.* 2015, Merceron *et al.* 2016). We begin

here with the premise that understanding differences within species will assist in managing the effects of broader sampling protocols and so allow for collection and analysis of larger samples. In addition, known differences in diet within species, particularly geographic differences, are detectable by DMTA (e.g., Scott *et al.* 2009, Merceron *et al.* 2010, Burgman *et al.* 2016). Incorporating intraspecific differences might thus enable more precise interpretations of diet for extinct taxa.

To date, intraspecific variability in DMTA has been poorly investigated. This is in contrast to earlier scanning electron microscopy (SEM) based microwear, where appraisals of intraspecific, or even intra-individual variation were common. Such analyses included investigation of differences between facets and teeth (e.g., Grine 1986, Robson and Young 1990, King *et al.* 1999b), taphonomic factors (e.g., Gordon 1983, Puech 1984, King *et al.* 1999a), sex (e.g., Gordon 1982, Ungar 1994, King *et al.* 1999a), ontogeny (e.g., Gordon 1984, Bullington 1988, Pérez-Pérez *et al.* 1999) and geography (e.g., Teaford and Robinson 1989, Teaford and Runestad 1992, Rafferty *et al.* 2002). Indeed, the results of this work likely underpinned the narrow sampling protocols of DMTA today (e.g., Scott *et al.* 2006, DeSantis *et al.* 2013, Calandra and Merceron 2016), despite having not yet been demonstrated as applicable in a DMTA framework. However, given the lack of inter-observer error in DMTA, which is found existing in low magnification (Semprebon *et al.* 2004, Mihlbachler *et al.* 2012) and SEM based microwear (Grine *et al.* 2002, Galbany *et al.* 2005, Estebaranz *et al.* 2009), DMTA may in fact be better placed to investigate such intraspecific questions. Understanding differences within species will in addition assist in determining the number of specimens required to detect a significant difference between samples given the relationship between power and distribution dispersion (Sokal and Rohlf 1969).

There are many potential sources of intraspecific variation in microwear texture patterns (table 4.1). A number of these can be considered in relation to a spatial hierarchy of scan

location. At the smallest scale, differences between facets—regions of teeth which occlude in "Phase II" (Kay and Hiiemae 1974)—seem a likely source of variation given the different role each facet plays in food breakdown (Evans and Sanson 2003). Only a few DMTA studies have analysed differences between facets (e.g., Krueger *et al.* 2008, Calandra *et al.* 2016a), or between enamel and dentine (Haupt *et al.* 2013). At the next level, individual teeth, even adjacent molars within the molar arcade, vary subtly in the way they interact with food (Ungar 2010). Comparing DMTA data from different tooth positions has been undertaken in: canids, where significant differences were found between teeth (Ungar *et al.* 2010); ungulates, where differences along the tooth row were only found in non-cud chewing taxa (Schulz *et al.* 2010); and primates, where no differences were found between P3 and M1 teeth (Daegling *et al.* 2011). Upper molars sit in the static maxilla, and lower molars in the mobile dentary and this difference in the physics of occlusion, combined with considerable differences between upper- and lower-molar morphology (Ungar 2010), may also effect microwear patterns. To date, such difference have only been assessed in equids (Schulz *et al.* 2010), despite sampling across upper and lower molars being common practice, even when other variables like tooth position and facet position are controlled (e.g., Ungar *et al.* 2007, Souron *et al.* 2015, Calandra *et al.* 2016b).

Natural variation in diet, particularly where species have large or heterogeneous ranges, is likely to impact microwear. DMTA studies have considered geographical variation in pigs (Souron *et al.* 2015), rodents (Burgman *et al.* 2016), voles (Calandra *et al.* 2016a, Calandra *et al.* 2016b), deer (Merceron *et al.* 2010), primates (Shapiro 2015), lemurs (Scott *et al.* 2009) and humans (e.g., Krueger and Ungar 2010, Schmidt *et al.* 2015, Calandra and Merceron 2016). Similar variation in diet across seasons has also been considered through DMTA (Merceron *et al.* 2010, Calandra *et al.* 2016a, Calandra *et al.* 2016b).

Table 4.1: Intraspecific factors that may affect DMTA data within species. * indicates factors considered in this study.

Factor	Type of Factor	Justification
Age*	Taphonomy	Deterioration of surfaces over time (DeSantis *et al.* 2014)
Element*	Sampling Location	Morphological differences between upper/lower molars (Schultz *et al.* 2010)
Facet*	Sampling Location	Roles of individual facets in food processing (Calandra *et al.* 2016b)
Geography*	Individual variation	Dietary variability across different ecosystems (Merceron *et al.* 2010)
Moulding material	Data Collection	Differences between moulding materials in replicating teeth (Goodall *et al.* 2015).
Profiler used*	Data Collection	Differences in microscope parameters (Arman *et al.* 2016)
Season	Individual variation	Annual changes in weather and flora (Merceron *et al.* 2010)
Specimen*	Individual variation	Sum of intra-individual variation (Merceron *et al.* 2010)
Tooth Position*	Sampling Location	Tooth-food interactions differ by location (Schultz *et al.* 2010)
Wear*	Individual variation	Worn teeth alter way in which food is broken down (McArthur and Sanson 1988)

As teeth are worn, they become less able to break down foods, and it is common for an animal to alter its diet or chew foods longer to accommodate such changes, e.g., for koalas (Lanyon and Sanson 1986), deer (Kubo and Yamada 2014), lemurs (Cuozzo *et al.* 2010), and kangaroos (McArthur and Sanson 1988). Whether there is a direct link between gross wear and DMTA data has so far only been considered in Mangabeys (*Cerecebus atys*) (Daegling *et al.* 2011), but the possibility that macroscopic wear will influence microwear either by reflecting changes in diet or by modifying the geometry of physical interactions which produce microwear has yet to be fully tested. A trial study however found no correlation between a number of topographic variables and DMTA data (Wimberly *et al.* 2016).

A broad source of potential variation in DMTA data is the geological age of a specimen. Most DMTA studies compare modern with palaeontological specimens to allow dietary inference. Palaeontological specimens however undergo a number of taphonomic processes, such as burial and deterioration, as well as post-deposional excavation and stabilisation

(Lyman 1994), all of which potentially alter the microwear signature present and have largely not been tested for in DMTA studies to date, with one exception (DeSantis and Haupt 2014).

Two recently-documented sources of variation in DMTA data are linked to data collection rather than biological factors. Differences have been noted between different confocal profilers used in DMTA data collection, and while a procedure exists to limit its effects, the process does not remove all differences between profilers (Arman *et al.* 2016). Some types of moulding material used to cast specimens have also been shown to affect data collected (Goodall *et al.* 2015).

A final intraspecific factor to consider is the specimen itself. All specimens differ somewhat from one another, notably along many of the lines mentioned above (e.g., *wear*, *ecoregion*). Which of these multiple factors considered for each specimen is significant, or most significant, can be difficult to determine. Moreover there may be important intraspecific factors that have yet to be considered. Considering variation between specimens then allows us to consider differences between individuals free of any over-arching theories regarding why they may differ.

Here we investigate within-species variability by comparing eight factors (and four reclassification of factors) likely to affect DMTA data. Macropodoids (kangaroos) were used to study these differences as different species vary along a clear dietary spectrum from browsing dicot-leaf-eating to grazing grass-eating (Arman and Prideaux 2015). Intraspecific factors were considered both within species, as well as in a larger multi-species dataset to determine whether variability within species is greater than dietary differences between species.

Intraspecific variation, where present, was then incorporated into specific models of DMTA data. To alleviate the risk of over-modelling through inclusion of redundant or non-

informative factors, models were developed through generalised linear mixed modelling (GLMM) (Zuur *et al.* 2009) and model selection criteria. This method allows factors to be modelled as fixed or random, as well as hierarchical structures of factors (Zuur *et al.* 2009), so is well suited to data with interspecific and intraspecific variance components. Models are compared by considering the r^2 for the model which describe how well the model fits with the DMTA data (Zuur *et al.* 2009), as well as Akaike's Information Criterion (AIC), which indicates the balance between each model's explanatory power and complexity in reference to the model's ability to fit the data (Burnham and Anderson 2003). Finally, the procedure utilises cross-validation, where the input dataset is split into development and testing subsets to allow an independent measure of the model fit (Osten 1988).

4.3. Methods

4.3.1. Casting procedures

Specimens were cleaned and cast using procedures that have become standard for DMTA (Scott *et al.* 2006), because casts retain high fidelity to the original tooth surfaces (Goodall *et al.* 2015). Teeth were cleaned using acetone, surfaces molded with President's Jet Plus polyvinylsiloxane Regular Body Dental Impression Material (Coltene-Whaledent), and casts were made using clear Epotek 501 epoxy resin and hardener (Epoxy Technologies). Modern specimens were accessed at: the Australian Museum, Sydney; Museum of Central Australia, Alice Springs; Museum Victoria, Melbourne; Queensland Museum, Brisbane; South Australian Museum, Adelaide; and Western Australian Museum, Perth. Palaeontological specimens representing both extinct and extant taxa are from the Main Fossil Chamber of Victoria Fossil Cave, Naracoorte, South Australia, and are housed at the South Australian Museum. Species sampled can be found in table 4.2, and have been chosen because they encompass a range of diets (Arman and Prideaux 2015) and tooth morphologies (Long *et al.* 2002).

Table 4.2: Species sampled for this study. * indicates palaeontological specimens**.** Niche and Average Grass Intake from (Arman and Prideaux 2015), and inferred for *Procoptodon gilli* based on morphology following (Prideaux 2003).

Species	Common Name	Niche	Average Grass Intake (%)	*N* specimens	*N* scans
Dendrolagus lumholtzi	Lumholtz's Tree-kangaroo	Small-bodied arboreal browser	Low	15	110
Macropus dorsalis	Black-striped Wallaby	Small-bodied grazer	93	30	106
Macropus robustus	Common Wallaroo	Large-bodied grazer	88	139	447
*Macropus rufogriseus**	Red-necked Wallaby	Small-bodied mixed feeder	27	19	51
Thylogale stigmatica	Red-legged Pademelon	Small-bodied mixed feeder	47	42	167
Thylogale thetis	Red-necked Pademelon	Small-bodied mixed feeder	Intermediate	32	128
*Procoptodon gilli**	extinct short-faced kangaroo	Large-bodied browser	Low	9	90
Wallabia bicolor	Swamp Wallaby	Small-bodied mixed feeder	47	67	276

4.3.2. Data acquisition

Specimens were scanned using two confocal profilers: a Sensofar Plμ "Connie" at the University of Arkansas, and a Sensofar Plμ NEOX "Bruce" at Flinders University. Median values of the four 102 x 139 μm^2 subscans taken on Connie were used to allow comparison with the larger 242 x 181 μm^2 scans from Bruce (following Arman *et al.* 2016). To minimise differences between profilers, the Soft Filter data processing template of Arman *et al.* (2016) was used on all scans in SensoMAP 7.1.2.7288 (Digital Surf). Data collected consisted of the two most commonly used SSFA and STA algorithms. The SSFA algorithms used were Area-scale fractal complexity (*Asfc* or 'complexity'), considering 71 scales from 0.02–100 μm, and Exact-proportion Length-scale anisotropy of relief (*epLsar* or 'anisotropy') at a scale of 2 μm (Scott *et al.* 2006). STA algorithms used were developed interfacial area ratio (*Sdr*), and void volume of the valleys (*Vvv*) at a material ratio of 80%. These algorithms together constitute those that have been most commonly used as well as having been previously successful at

identifying differences between dietary groups (e.g., Scott *et al.* 2006, Schulz *et al.* 2010, Calandra *et al.* 2016a). All data were collected using Sfrax 1.0.11.882 (Surfract Inc.), and SensoMAP 7.1.2.7288 (Digital Surf) and analysed using the R computing environment (R Core Team 2012).

4.3.3. Analysis

To determine how sample size influenced estimates of intraspecific variability, large samples of the grazing *Macropus robustus* and the mixed-feeding *Wallabia bicolor* were analysed by subsampling. These taxa were chosen because they were both sampled extensively and have distinct diets (Arman and Prideaux 2015). Data for *Dendrolagus lumholtzi*, *M. dorsalis* and *M. rufogriseus* were also included in subsampling analyses to investigate factors that could not be adequately sampled in *M. robustus* and *W. bicolor*. *Specimen* however could not be investigated by subsampling as no individual specimen had wear present on enough teeth to attain a sufficient sample size. The effect of sample size on the precision of DMTA data was estimated with a bootstrap approach where, for each sample size, 10,000 subsamples were drawn (with replacement) and the median and standard deviation for each computed (see SI 2). Datasets were then controlled to sample a single level of each factor (e.g., *wear* stage 2 only), and the subsample mean and standard deviation recalculated. In addition, datasets comprising random samples of undifferentiated (e.g., all data) and those controlled by factors were constructed of sample sizes 1–50. These were then compared to determine at what sample size the species could be statistically differentiated. This was undertaken using Kruskal–Wallis tests necessitated by non-normal distributions, particularly at low sample sizes.

Prior to further analysis, normality of datasets was evaluated with histograms and quantile–quantile plots (see SI 1). Transformations were used to normalise data for analysis, and typically involved natural-log transformations for *Asfc*, *Sdr* and *Vvv*, and square-root

transformations for *epLsar*, though the full analysis used transformation of *epLsar* ^ 0.7 following boxcox log-likelihood plots (see SI 1).

Twelve intraspecific factors were considered: *specimen, element, tooth, facet, facet side, facet face, facet side + face, wear, coarse wear, microscope, age,* and *ecoregion*. *Specimen* used the unique specimen numbers of each individual sampled to encapsulate all inter-individual variation. *Element* considered whether the tooth scanned was an upper or lower molar. *Tooth* considers which tooth (dp3, m1, m2, m3, m4 or mx=unknown) was scanned. *Facet* described which of the wear facets was scanned. *Facet* numbering for macropodoids has not been previously described, so has been transcribed from the general mammalian pattern (Kay and Hiiemae 1974), in figure 4.1. Larger facets normally had a higher likelihood of preserving dental microwear, meaning that sample sizes varied depending on facet size. Additionally, the bilophodont condition of macropodoids results in the repetition of functionally homologous facets between lophs. Facets were thus reclassified in three ways based on the upper molar to establish a more simplistic classification that may better delineate inter-facet differences. *Facet side* reclassified facets based on position relative to the buccal or lingual margin of the tooth midline. *Facet face* specifies whether the facet exists on the anterior or posterior loph face. *Facet side + face* combines these anterior-posterior and buccal-lingual positional information into a single factor. *Wear* indicates how worn the tooth being scanned was, based on a dentine exposure scale established by McArthur and Sanson (1988), with modifications made to accomodate wear patterns in sthenurine (short-faced) kangaroos (table 4.3). The decreasing size of lophs as teeth are worn resulted in relatively low sample sizes for heavily, compared with less worn teeth. To address this, *coarse wear* undertook comparison with wear stages of four or greater combined. *Microscope* considers which of the two microscopes was used to scan the tooth. *Age* considered whether specimens were modern or palaeontological to determine whether taphonomic factors effected microwear.

Ecoregion considered the geographic origin of modern specimens. Ecoregions were identified using World Wildlife Federation Terrestrial Ecoregions (Olson *et al.* 2001), though the names of individual ecoregions have been abbreviated for convenience. Ecoregion of each specimen was attained by utilising ecoregion GIS shapefiles available at <http://maps.tnc.org/gis_data.html> and locality data from museum specimen databases. These spatial datasets were merged in ArcMap 10.2 (Esri Inc.) to assign specimens to an *ecoregion* (see figure 4.2). Modern specimens without locality data and all palaeontological specimens were coded as 'N/A' and analysed as a separate group.

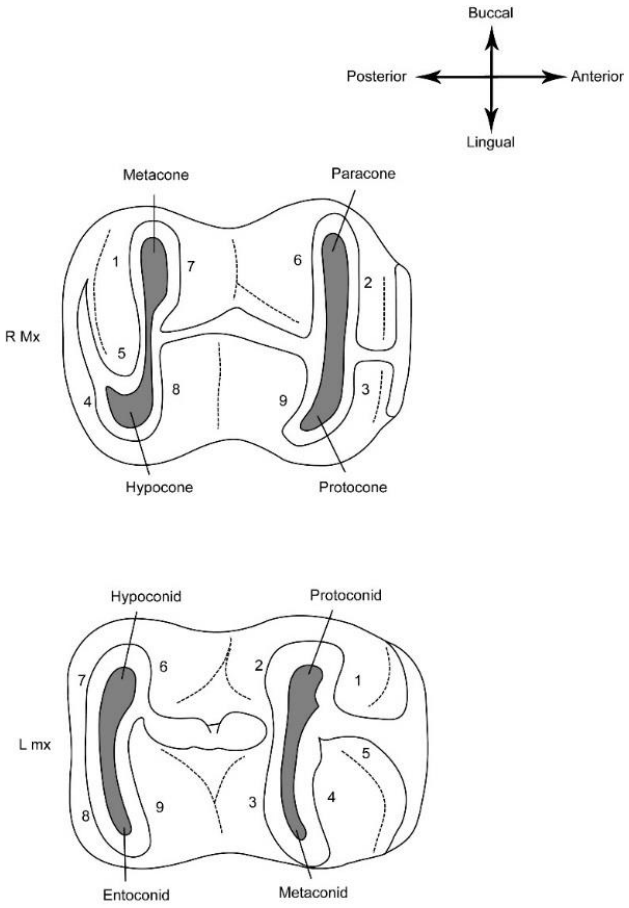

Figure 4.1: Macropodoid wear facets as transcribed from the pattern in primates (i.e. Kay and Hiiemae (1974)). Grey areas indicate exposed dentine, dotted lines indicate separation between facets or other areas of relief. *Macropus* sp. upper (above) and lower (below). Modified from Thenius (1989; figure 104).

Table 4.3: Successive molar-wear stages for sthenurine kangaroos.

Wear Stage	Description
0	No wear
1	Development of minor wear facets near lophids
2	Loss of some lophid crenulations
3	Loss of more crenulations, including complete loss of some
4	Dentine exposed on buccal cusps
5	Dentine exposed on buccal and lingual cusps
6	Connection of dentine between buccal and lingual cusps

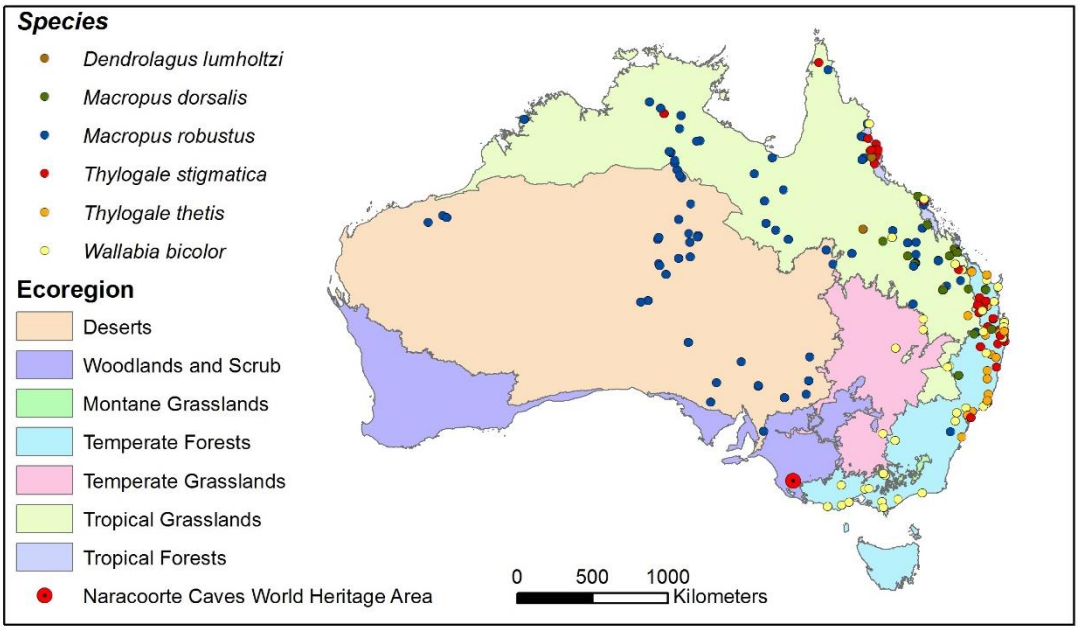

Figure 4.2: Ecoregion and locality data of specimens used in this study. Ecoregions abbreviated from World Wildlife Federation Terrestrial Ecoregions (Olson *et al.* 2001).

To assess intraspecific factors in isolation, within-species analyses effectively remove or minimise the interspecific dietary signal obfuscating the influence of other factors. For some factors (*tooth, facet, facet side, facet face, facet side + face, specimen, wear* and *coarse wear*), this was undertaken across eight single-species datasets. Analysis of *ecoregion* was limited to the six species sampled from modern collections. Due to sample-size limitations, comparisons of *microscope* were limited to *D. lumholtzi* and *M. dorsalis* and comparison of *element* restricted to *M. robustus* and *M. rufogriseus*. *Age* could not be compared within species due to differences in species present between modern and palaeontological

specimens. However, *Age* and indeed all factors were compared across an additional dataset of all species present to determine if intraspecific variation was outweighed by differences between species. T-tests were used to compare factors with two levels (e.g., *microscope*) and ANOVA tests for factors with three or more levels (e.g., *facet*).

More complex models were then constructed to determine if and how dietary differences may be better modelled by including intraspecific factors. Models were compared using generalised linear mixed modelling (GLMM) (Zuur *et al.* 2009), using the script provided in SI 2. This calculated deviance, AIC, r^2, and cross-validated r^2 (cvr^2) for each model generated. AIC and cvr^2 were predominantly used to compare models with a lower AIC, and high cvr^2 indicating an improvement on earlier models.

Model construction began with the factor *specimen* alone as a random factor to encapsulate inter-individual variation. This also eliminates pseudoreplication when multiple scans of an individual are included. Including *specimen* is also justified given the considerable variation accounted for by the *specimen* models which account for > 75% of variation found in comparison to final models for all variables (see table 4.6). The second model consisted of *specimen* + *species*, as species represents the essential dietary groups being compared. Additional sets of models were constructed in a 'bottom up' approach, where each subsequent model included a different intraspecific factor, and each of these models compared. The model which produced the lowest AIC score was then used as the base for the subsequent set of models and the remaining factors added, recursively. This process continued until no improvement was seen in AIC score. A final set of models was then constructed comparing each of best models from each set. Of these, the model with the greatest cvr^2 was considered the best candidate for each variable.

4.4. Results

4.4.1. Summary

Within-species variation can be detected for each of the factors investigated across all four response variables. Differences within species however are outweighed by differences between species. By incorporating many of the intraspecific factors into more complex models, we can improve the ability to discern differences between dietary groups and species. Data collected can be found in SI 3.

4.4.2. Subsampling

Subsampling demonstrates that large samples are required for species to achieve low variation in both SSFA and STA variables (see SI 4), though this is decreased when intraspecific differences are controlled for. Statistical differentiation between the species considered here however is possible at low sample sizes (table 4.4). When intraspecific factors are controlled, most analyses identified significant differences at lower sample sizes, though this varied between factors and DMTA variables.

4.4.3. Intraspecific Analyses

Removing the dietary signal by considering each of the eight taxa in isolation revealed significant differences between samples for most factors (table 4.5). When considered across all species, all factors except *tooth* and *specimen* also showed significance for at least one of the four measures used. Despite measuring different elements of surface texture in very different ways, the different DMTA measures all demonstrated similar results, even when comparing SSFA and STA variables.

4.4.4. Modelling

For all variables, models improved in both AIC (where a lower score indicates an improved model) and cvr^2 when intraspecific factors were included. Model progression is illustrated in figure 4.3, and shows model development for all factors begins to plateau at four factors (e.g.,

species + specimen + factor1 + factor2), and final models contained between four and seven factors. Alongside *species* and *specimen*, *facet* was the only factor contained in all final models. *Microscope* and *ecoregion* also featured in the final model for all variables except for *epLsar.* All models compared can be seen in SI 3, and final models compared can be seen in table 4.6. Final models improve our ability to discern between species as is demonstrated in figure 4.4.

Table 4.4: Sample size required to differentiate between selected species as determined by subsampling. Factors controlled by level with highest available samples: none = undifferentiated, Tooth = M2, Ecoregion = Tropical Grasslands, Wear = stage 2, Facet = 6, Facet Side = buccal, Facet Face = posterior, Facet Side + Face = buccal posterior, Tooth + Facet = M2 facet 6, Element = upper, Microscope = 'Bruce'. Tooth + Facet = M2 + facet 6 used to demonstrate the utility of controlling for tooth and facet as is common (e.g., Scott et al. 2006, Stynder et al. 2012, Merceron et al. 2014). Kruskal–Wallis tests used to differentiate taxa, with three consecutive significant (p = <0.05) results considered to indicate a reliable stage of differentiation. 'N/A' indicates reliable differentiation not found for the available sample size.

Controlled Factor	***Asfc***	***epLsar***	***Sdr***	***Vvv***
Macropus robustus vs *Wallabia bicolor*				
None	9	31	7	15
Tooth	4	21	23	30
Facet	9	12	9	11
Facet Side	11	7	8	18
Facet Face	17	10	13	14
Facet Side + Face	7	12	5	8
Wear	9	N/A	6	11
Tooth + Facet	14	7	11	N/A
Ecoregion	9	8	9	15
Macropus robustus vs *Macropus rufogriseus*				
None	6	4	7	11
Element	6	6	4	6
Dendrolagus lumholtzi vs *Macropus dorsalis*				
None	N/A	7	11	7
Microscope	7	7	7	10

Table 4.5 (page 134–135): Intraspecific differences in DMTA data resulting from ANOVAs (*Facet, Facet side + Face, Tooth, Wear, Coarse Wear, Ecoregion* and *Specimen*) or T-test (*Facet Side, Facet Face, Element, Age, Microscope*). Each column represents a single-species dataset, while 'ALL' refers to differences found across the entire dataset. Area-scale fractal complexity (*Asfc*); Exact-proportion Length-scale anisotropy of relief (*epLsar*); developed interfacial area ratio (*Sdr*), and void volume of the valleys at a material ratio of 80% (*Vvv*). All variables normalised prior to analysis (see SI1). Significant (p <0.05) differences in bold.

Measure	Factor	*Dendrolagus lumholtzi*	*Macropus dorsalis*	*Macropus robustus*	*Macropus rufogriseus*	*Procoptodon gilli*	*Thylogale stigmatica*	*Thylogale thetis*	*Wallabia bicolor*	**ALL**
Asfc	Specimen	**0.016**	**<0.001**	**<0.001**	**<0.001**	0.059	**<0.001**	**<0.001**	**<0.001**	0.160
epLsar	Specimen	0.060	**0.038**	0.137	0.608	**0.031**	0.317	0.134	0.682	0.487
Sdr	Specimen	**0.010**	**<0.001**	**<0.001**	**<0.001**	0.052	**<0.001**	**<0.001**	**<0.001**	0.117
Vvv	Specimen	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.057	**<0.001**	**0.018**	**<0.001**	0.427
Asfc	Tooth	0.453	**0.038**	0.481	0.700	0.051	0.187	0.089	**0.019**	0.177
epLsar	Tooth	0.499	0.618	0.263	0.597	0.059	0.597	0.920	0.538	0.502
Sdr	Tooth	0.272	0.079	0.402	0.938	**0.041**	0.233	0.089	**0.007**	0.117
Vvv	Tooth	0.117	0.213	0.565	0.836	0.139	0.314	0.379	**0.011**	0.427
Asfc	Facet	**0.05**	0.147	0.075	0.791	**<0.001**	**0.001**	0.322	0.973	0.051
epLsar	Facet	0.622	0.378	**<0.001**	**0.012**	0.436	**0.002**	0.254	0.199	**<0.001**
Sdr	Facet	0.112	**0.027**	**0.036**	0.830	**<0.001**	**0.003**	0.346	**0.026**	0.062
Vvv	Facet	0.407	**<0.001**	**0.009**	0.777	**0.009**	0.185	0.596	**0.124**	**0.027**
Asfc	Facet Side	0.093	0.969	0.759	0.502	**<0.001**	0.462	0.473	0.147	0.274
epLsar	Facet Side	0.250	0.056	**<0.001**	**0.001**	0.981	0.437	0.383	**<0.001**	**<0.001**
Sdr	Facet Side	0.075	0.366	0.408	4.424	**<0.001**	0.395	0.421	0.162	0.127
Vvv	Facet Side	0.566	**<0.001**	0.527	0.381	**0.003**	0.928	0.352	0.681	0.167
Asfc	Facet Face	**<0.001**	**0.002**	0.556	0.296	**0.007**	**0.001**	0.087	0.349	0.123
epLsar	Facet Face	0.498	**0.032**	**<0.001**	0.079	0.367	**0.003**	0.190	0.586	**<0.001**
Sdr	Facet Face	0.059	**0.004**	0.184	0.222	**0.004**	**0.004**	0.195	0.076	0.270
Vvv	Facet Face	0.090	**<0.001**	**0.015**	0.110	**0.004**	0.258	0.355	0.073	0.257
Asfc	Facet Side + Face	**0.018**	**0.041**	0.510	0.357	**<0.001**	**0.007**	0.385	**0.035**	**0.037**
epLsar	Facet Side + Face	0.358	0.086	**<0.001**	**0.001**	0.946	**0.028**	0.298	**0.001**	**<0.001**
Sdr	Facet Side + Face	**0.045**	**0.005**	0.184	0.368	**<0.001**	**0.014**	0.489	**0.010**	0.365
Vvv	Facet Side + Face	0.156	**<0.001**	**0.041**	0.289	**0.004**	0.333	0.574	0.113	0.348
Asfc	Wear	0.551	0.063	**0.004**	0.069	0.334	**0.006**	**<0.001**	0.238	**<0.001**
epLsar	Wear	**0.022**	0.830	0.740	0.134	0.264	**0.001**	0.188	0.624	0.335
Sdr	Wear	0.656	0.199	**0.04**	0.154	0.167	**0.003**	**<0.001**	0.175	**<0.001**
Vvv	Wear	0.679	0.371	0.466	0.216	0.167	**0.003**	**0.006**	0.469	**<0.001**
Asfc	Coarse wear	0.551	0.214	0.449	0.069	0.334	**0.006**	**<0.001**	0.238	**0.017**
epLsar	Coarse wear	**0.022**	0.743	0.442	0.134	0.264	**0.001**	0.188	0.624	0.081
Sdr	Coarse wear	0.656	0.325	0.580	0.154	0.167	**0.003**	**<0.001**	0.175	**0.006**
Vvv	Coarse wear	0.679	0.277	0.318	0.216	0.167	**0.003**	**0.007**	0.469	**<0.001**

Table 4.5 (continued)

Measure	Factor	*Dendrolagus lumholtzi*	*Macropus dorsalis*	*Macropus robustus*	*Macropus rufogriseus*	*Procoptodon gilli*	*Thylogale stigmatica*	*Thylogale thetis*	*Wallabia bicolor*	**ALL**
Asfc	Microscope	**<0.001**	**0.001**	N/A	N/A	N/A	N/A	N/A	N/A	**0.041**
epLsar	Microscope	**0.041**	0.396	N/A	N/A	N/A	N/A	N/A	N/A	**<0.001**
Sdr	Microscope	**0.012**	0.337	N/A	N/A	N/A	N/A	N/A	N/A	**<0.001**
Vvv	Microscope	**<0.001**	**<0.001**	N/A	N/A	N/A	N/A	N/A	N/A	**<0.001**
Asfc	Age	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**<0.001**
epLsar	Age	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.706
Sdr	Age	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**<0.001**
Vvv	Age	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**<0.001**
Asfc	Ecoregion	0.242	**<0.001**	**<0.001**	N/A	N/A	0.176	**0.015**	**<0.001**	**<0.001**
epLsar	Ecoregion	0.699	0.678	0.524	N/A	N/A	0.267	0.388	0.086	**0.002**
Sdr	Ecoregion	0.139	**<0.001**	**<0.001**	N/A	N/A	0.098	**0.013**	**<0.001**	**<0.001**
Vvv	Ecoregion	**<0.001**	0.165	**<0.001**	N/A	N/A	**0.011**	**0.043**	**0.019**	**<0.001**

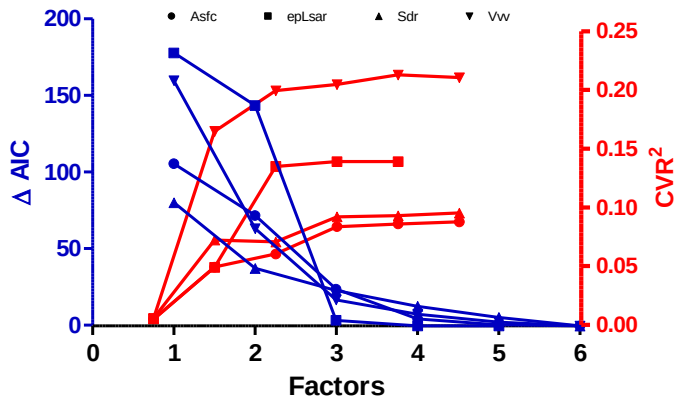

Figure 4.3: Model improvement with additional factors. Factor 1 = *Specimen*; factor 2; *Species,* additional factors differ between variables (see table 4.6). Δ AIC: difference in AIC from lowest model for each variable, cvr^2: cross-validated regression of model with independent testing dataset. Area-scale fractal complexity (*Asfc*); Exact-proportion Length-scale anisotropy of relief (*epLsar*); developed interfacial area ratio (*Sdr*), and void volume of the valleys at a material ratio of 80% (*Vvv*). All variables normalised prior to analysis (see SI1).

Table 4.6: Comparison of final models. AIC: Akaike's Information criterion, Δ AIC: difference in AIC from lowest calculated for each variable, r^2: regression of model with development dataset, cvr^2: cross-validated regression of model with independent testing dataset. Area-scale fractal complexity (*Asfc*); Exact-proportion Length-scale anisotropy of relief (*epLsar*); developed interfacial area ratio (*Sdr*), and void volume of the valleys at a material ratio of 80% (*Vvv*). Specimen modelled as a random factor. All variables normalised prior to analysis (see SI1). Models ordered by cvr^2.

	Model	AIC	Δ AIC	R^2	CVR^2
Asfc	Species + Specimen + Microscope + Ecoregion + Tooth + Facet + Element	1704.992	0.000	0.555	0.089
	Species + Specimen + Microscope + Ecoregion + Tooth + Facet	1705.668	0.676	0.553	0.087
	Species + Specimen + Microscope + Ecoregion + Tooth	1709.327	4.335	0.545	0.085
	Species + Specimen + Microscope	1728.137	23.145	0.538	0.059
	Species + Specimen	1774.118	69.125	0.512	0.047
	Specimen	1806.454	101.461	0.516	-0.001
epLsar	Species + Specimen + Facet + Coarse Wear	-9607.274	0.000	0.220	0.146
	Species + Specimen + Facet + Coarse Wear + Age	-9607.274	0.000	0.220	0.146
	Species + Specimen + Facet	-9603.725	3.549	0.216	0.141
	Species + Specimen	-9469.442	137.832	0.132	0.047
	Specimen	-9436.676	170.599	0.175	-0.001
Sdr	Species + Specimen + Microscope + Ecoregion + Tooth + Facet	1905.959	0.000	0.560	0.098
	Species + Specimen + Microscope + Ecoregion + Tooth + Facet + Age	1905.959	0.000	0.560	0.098
	Species + Specimen + Microscope + Ecoregion + Tooth	1911.353	5.393	0.551	0.095
	Species + Specimen + Microscope + Ecoregion	1918.396	12.437	0.543	0.094
	Species + Specimen	1941.983	36.024	0.535	0.072
	Species + Specimen + Microscope	1928.226	22.266	0.545	0.071
	Specimen	1983.067	77.107	0.540	-0.001
Vvv	Species + Specimen + Microscope + Facet + Ecoregion	1724.431	2.369	0.528	0.227
	Species + Specimen + Microscope + Facet + Ecoregion + Tooth	1722.062	0.000	0.531	0.224
	Species + Specimen + Microscope + Facet	1729.402	7.340	0.528	0.218
	Species + Specimen + Microscope	1738.549	16.488	0.517	0.212
	Species + Specimen	1783.014	60.952	0.506	0.174
	Specimen	1875.357	153.295	0.522	-0.001

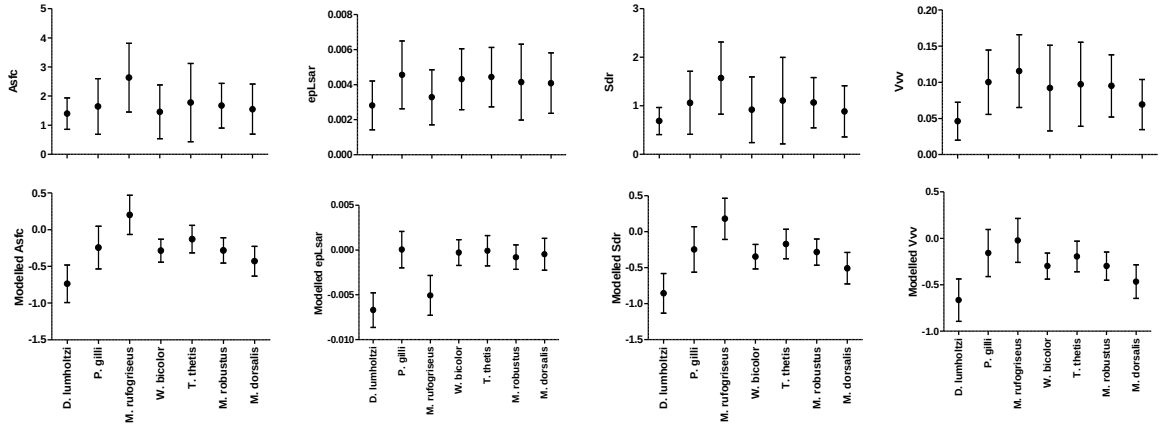

Figure 4.4: Interspecific differences (mean and 95% confidence intervals) in DMTA data. Raw data above, and modelled data (developed through GLMM) below. Species in each plot were ordered by dietary classification (Arman and Prideaux 2015), with browsers left to grazers right, and *Procoptodon gilli* placed roughly where this taxon would be expected to fall based on morphology. *Thylogale stigmatica* used as dummy level for all modelled data. Plots left to right: Area-scale fractal complexity (*Asfc*); Exact-proportion Length-scale anisotropy of relief (*epLsar*); developed interfacial area ratio (*Sdr*), and void volume of the valleys at a material ratio of 80% (*Vvv*). All variables normalised prior to analysis (see SI1).

4.5. Discussion

DMTA data vary within species due to intraspecific variability and precisely where and how sampling is undertaken. Where this variability has been considered at all in previous work, it has been by 1) controlling for variability, which severely limits palaeontological sampling, or 2) testing for variation independent of dietary analyses, which inherently isolates intraspecific from interspecific comparisons. Here we argue for incorporating variability through broader sampling, and modelling of intraspecific factors within dietary analyses, which can improve analytical outcomes, facilitating rather than obfuscating dietary resolution.

4.5.1. Interspecific differences in macropodid microwear

The GLMM modelling improved our ability to recognise dietary differences in the microwear data collected such that the models uncover much clearer differences between species (figure 4.4). Similar results for models of *Asfc, Sdr* and *Vvv* supports the idea that these variables may capture similar aspects of surface texture (Schultz et al. 2010, Calandra et al. 2012). Here, we focus on *Asfc* (complexity) because it is the best understood of the three variables (Calandra and Merceron 2016).

Modern specimens of *Thylogale thetis* (Red-necked Pademelon), *Macropus robustus* (Common Wallaroo) and *M. dorsalis* (Black-striped Wallaby), and to a lesser degree *Wallabia bicolor* (Swamp Wallaby) exhibit a trend toward decreasing complexity (and *Sdr* and *Vvv*) with increasing grass intake, as would be expected based on patterns in primates and ungulates high (e.g., Scott *et al.* 2006, DeSantis *et al.* 2013, Calandra and Merceron 2016). Modern specimens of the obligate browser *Dendrolagus lumholtzi* (Lumholtz's Tree-kangaroo) though have low complexity, in contrast to most browsers where complexity is high high (e.g., Scott *et al.* 2006, DeSantis *et al.* 2013, Calandra and Merceron 2016). This result may be due to the arboreal nature of *D. lumholtzi*, particularly given the ongoing speculation that it may be sand or grit, rather than food itself that is reflected in DMTA data (e.g., Ryan 1979, Lucas et al. 2013, Calandra and Merceron 2016).

The extinct short-faced kangaroo *Procoptodon gilli* exhibits a wider range in complexity than most other species, but its overlap principally with extant mixed-feeding taxa (figure 4.4) suggests a mixed diet. Fossil specimens of *Macropus rufogriseus* (Red-necked Wallaby) exhibit the highest complexity values, which may suggest a browsing diet in the past, in contrast to its modern mixed-feeding diet (Arman and Prideaux 2015).

Interpreting the GLMM modelled data for anisotropy (*epLsar*) is difficult. *Dendrolagus lumholtzi* and *M. rufogriseus* exhibit much lower anisotropy values than other species, consistent with browsing taxa, but these species cannot be entirely differentiated from each other.

Greater coverage of the dietary spectrum in macropodids is needed to refine these preliminary results. From the perspective of dietary representation, it will be necessary to investigate non-arboreal as well as additional arboreal browsing species to test the idea that *Dendrolagus lumholtzi* has low-complexity microwear because it infrequently ingests grit. From a modelling standpoint, greater coverage of the dietary spectrum amongst modern and fossil taxa would help fine tune our understanding of how microwear is linked to diet in macropodids. Other factors that might be possible to explore under a broader sampling regime such as feeding height potentially open new avenues for understanding ecological drivers of microwear patterns.

4.5.2. GLMM in DMTA

The GLMM modelling process undertaken herein improved our ability to recognise dietary trends in the DMTA data collected. This is demonstrated most clearly in figure 4.4, where the modelled data show much clearer delineation between species, and proportionally less variation than the raw DMTA data.

The final modelled data were arrived at through GLMM modelling. The benefits of this method are principally in its simplicity. While the mathematics of modelling can be difficult to comprehend, the fundamental methodology in its application are not. The tools of AIC and cvr^2 are particularly beneficial in this regard as simple means to compare and improve models. Rather than trying to rule out factors where significant variation cannot be demonstrated, models built following AIC comparisons will include factors with any

consistent variability, but only if they improve the predictive power of the model (Burnham and Anderson 2003). In addition, cross-validation allows a formal evaluation of the predictive capacity of a model, since model predictions are compared to a subset of the dataset not used for model fitting (Osten 1988). Using cvr^2 in GLMM thus decreases the likelihood to select a final model which is over-fit to the development dataset (Osten 1988).

Further forays into DMTA data modelling may be through considering interactions between variables, though preliminary trials suggest that such an approach is of little benefit unless a particular hypothesis is of interest in modelling. Alternatively, including other data in predictive modelling, particularly dietary data, may improve our ability to model DMTA data along dietary lines.

4.5.3. Comparisons to previous work

Most of the intraspecific factors investigated here have been shown elsewhere to be significant in DMTA, for example tooth position (Ungar *et al.* 2010), facet (Krueger *et al.* 2008), element (Schulz *et al.* 2010), geography (e.g., Teaford and Walker 1984, Ungar *et al.* 2012, Merceron *et al.* 2016), and profiler used (Arman *et al.* 2016). Moreover these results also accord with those seen in scanning electron microscopy based microwear, such as differences between teeth (Gordon 1982, Gordon 1984), facets (e.g., Gordon 1982, Gordon 1984, Teaford and Walker 1984), element (Pérez-Pérez *et al.* 1999), wear stages (King *et al.* 1999a), geography (Teaford and Runestad 1992), or taphonomic processes (King *et al.* 1999b, Pérez-Pérez *et al.* 1999).

The approach employed here contrasts to earlier work on how to incorporate variability within species to maximise the ability to discern differences between species. Most DMTA studies control factors to limit intraspecific variability (e.g., Scott *et al.* 2006, Stynder *et al.* 2012, Merceron *et al.* 2014). Controlling for, rather than incorporating factor variability

limits analysis by disallowing intraspecific variability to be incorporated into dietary analyses.

Factors such as *wear* and *ecoregion*, are intrinsically linked to inter-individual variation and so incorporating their variation is likely to improve analytical outcomes, as these factors cannot be practically controlled for in most museum collections. However, for datasets where such factors cannot be scored, the high variability accounted for by specimen alone (table 4.5), suggests that much inter-individual variation can be accounted for just by including *specimen* as a random factor in GLMM models.

Some researchers have looked into intraspecific variability in combination with specific (e.g., Krueger *et al.* 2008, Ungar *et al.* 2010, Calandra *et al.* 2016a), or geographic analyses (e.g., Ungar *et al.* 2007, Scott 2012, Merceron *et al.* 2016). In these cases, however, the analyses were simple, and no comparative tools or modelling were used. Others have looked at intraspecific factors separately from specific analyses (e.g., Schulz *et al.* 2010, Haupt *et al.* 2013, DeSantis and Haupt 2014), or even split data by a factor that was shown to differ (Merceron *et al.* 2010). By separating intraspecific variation from dietary comparisons, these approaches are more analytically limited as they do not allow for models to incorporate intraspecific factors into dietary differentiation.

4.5.4. Intraspecific factors

Each of the factors investigated showed significant variation in the single-species analyses (table 4.4). Consideration of the same factors across the entire dataset however varied, and demonstrates that intraspecific variation can be masked in large multi-species datasets. The cause of variation for each of the factors is considered in detail in SI 5, and are the product of a range of biological and physical processes.

The influence of each factor varied considerably in the final models produced by GLMM. Alongside *species* and *specimen,* only 6 of the 12 intraspecific factors investigated were used in the final GLMM models. Of these, only *facet* featured in all the final models, supporting well-understood differences between facets (e.g., Gordon 1984, Krueger *et al.* 2008, Calandra *et al.* 2016a). *Microscope* and *ecoregion* also featured in three of four final models. Differences between microscopes appear to still plague studies conducted on different profilers, despite the recommended filtering being utilised to minimise such effects (Arman *et al.* 2016). Including *microscope* in modelling however should help alleviate remaining microscope-based variation and may be necessary for data sharing. The utility of *ecoregion* lies in the fact that many kangaroo species occupy extensive geographic distributions within which there is marked variation in rainfall, vegetation structure, etc. (Olson *et al.* 2001). Although the specifics of how these factors influence DMTA data are hard to determine, the general signature provided by *ecoregion* may be the best way to incorporate broad environmental factors into DMTA.

The factors *tooth, element,* and *coarse wear* also featured in final models, but not as universally as other terms. This may suggest that the effect of these factors are only relevant for some variables and are irrelevant or outweighed by variability elsewhere. The inclusion of factors related to scan location; *facet, tooth* and *element,* in the final model for complexity is also noteworthy, and suggests that where the scan is taken on an individual may be particularly important to control or manage for this variable.

Factors not featured in any model are also worth noting. *Age* did not feature in any final model suggesting no consistent impact of broad taphonomic alteration for the Victoria Fossil Cave material. The reclassifications *facet side, facet face,* and *facet side + face* featured in no final models suggesting that it is the particular *facet* not the approximate location on the tooth that effects DMTA data. In contrast, *wear* featured in no final models, but *coarse wear* was in

the final anisotropy model, suggesting a coarser classification to be more useful, likely because all of the high wear categories combined for this have occlusion largely compromised (McArthur and Sanson 1988).

Species and *specimen* were *prima facie* included in all final models, so their inclusion was not determined through GLMM like other factors. Including *species*, is of course necessary to consider dietary differences, however considering diet instead through a dietary classification (e.g., Arman and Prideaux 2015) could be similarly compared through GLMM. *Specimen* however was included as a random factor as required by GLMM (Zuur *et al.* 2009). We also do not want to evaluate the effect of each individual specimen as would be the case if *specimen* were a fixed effect. This means that to consider models without *specimen* would require either assigning another factor as random or utilising a different modelling method. No other factors however seem appropriate as random, and the inter-individual variation encapsulated by *specimen* as a random factor appears well justified. Importantly, *specimen* is an inherently simple factor to consider in future studies as this information is retained in standard DMTA data collection procedures.

The locality data used in *ecoregion* comparisons are also available for a large number of modern specimens, so focusing sampling on specimens for which locality is known may be the best way to prioritise extant DMTA data collection. Similar DMTA studies considering geography elsewhere (e.g., Merceron *et al.* 2010, Burgman *et al.* 2016, Calandra *et al.* 2016b) have focused upon identifying differences between specific regions of interest. In contrast however, the ecoregion data used here is a global dataset (Olson *et al.* 2001) and so undertaking similar analyses to those here on other taxa and regions should be straightforward as well as consistent.

It may be important to consider the factors identified herein in other mammalian groups to determine which factors are universal, rather than due to particularities in macropodoid mastication, diet or ecology. Beyond improving dietary comparisons, further investigation into these or other factors may also yield greater insight along alternate lines. For example, modelling focussed on differentiating *ecoregions* rather than *species* may yield inferences regarding past environments. Considering etiology of microwear by focusing models on differences between *facets* could also join more recent efforts (Krueger *et al.* 2008, Calandra *et al.* 2016a), to bring DMTA back to the roots of dental microwear, where scratches on teeth were first investigated to understand mastication and food processing (e.g., Ryder 1878, Butler 1952, Mills 1978).

4.5.5. DMTA variables

Three of the four measures used (*Asfc*,*Sdr* and *Vvv*), once modelled, produced similar interspecific plots (Figure 4.4). Where intraspecific differences were evident in the eight single-species datasets, they typically differed for more than one variable, and importantly the SSFA and ISO variables performed similarly.

Subsampling showed controlling for some factors (e.g., *facet*) to be particularly effective at distinguishing between taxa for all variables, regardless of whether they were SSFA or STA variables. Other factors were more effective for some variables than others (e.g., *Asfc* in comparison to *Vvv* for *ecoregion*). While this validates constrictive data collection, including such factors in data modelling effectively achieves the same outcome without restricting available sample size. The modelled effect of each factor (see SI 5) also yield some interesting possibilities, including most variation by *tooth* being focused on M4, which could be important to consider for sampling practices. In contrast, the modelled effects for facet showed the lowest variability for well-sampled facets, further demonstrating the importance of large sample sizes.

The final models determined through the model selection process also showed overwhelming similarity between the different variables. The factor *facet* featured in the final models for all four variables considered. Moreover the final models for *Asfc, Sdr* and *Vvv* all shared the factors *ecoregion* and *microscope,* and further the final models for *Asfc* and *Sdr* also share the factor *tooth.* Indeed the interspecific plots featured in figure 4.4 for *Asfc, Sdr* and *Vvv* are overwhelmingly similar, and suggests that once modelled, these variables may in fact be recording the same elements of surface texture.

A possibility for future work may be to consider the application of GLMM methods to other DMTA variables in both SSFA and STA. As seen here between *epLsar* and other variables, other SSFA variables are considered to capture different elements of diet or occlusion (Scott *et al.* 2006, Calandra and Merceron 2016). Indeed the low regression found for the final anisotropy model ($r^2 = 0.22$), suggests that much of the variation present is yet to be accounted for. Given the similarity between SSFA variables here, perhaps rather than modelling each separately, many DMTA variables could be modelled together to each contribute to a balanced dietary model, rather than having researchers attempt to weigh up the influence of the often > 20 variables utilised (e.g., Schulz *et al.* 2010, Calandra *et al.* 2016a).

4.6. Conclusions

Intraspecific variation in DMTA data has the potential to obfuscate analysis when not controlled for, but also offers the potential to refine differences between dietary groups. We show that all intraspecific factors investigated altered DMTA variables significantly. Rather than controlling for variation within species or individuals, we incorporated intraspecific variation into interspecific models. Generalised linear mixed-modelling allowed intraspecific variation to be incorporated into models, improving definition of dietary differences. A small number of factors; *facet, microscope* and *ecoregion* featured in the final models for most

variables, suggesting that these have the greatest ability to refine dietary analyses. In addition, including *specimen* as a random effect encapsulates inter-individual variation.

Sampling across rather than controlling for factors enables the effects of intraspecific variability to be taken into account. This is best achieved through modelling such as GLMM undertaken here, which provides a simple, objective method to compare various models. Sampling across a wider range of *teeth*, *facets* etc. also allows for larger samples to be attained, which is of particular utility in boosting sample sizes for palaeontological studies.

4.7. Acknowledgements

Thanks to Grant Gully for creating figure 4.1. Thanks to Sal Caporale and Sarah Livengood for assistance in scanning at the University of Arkansas. Thanks to Sandy Ingleby, Adam Yates, Karen Roberts, Heather Janetzki, David Stemmer, Kenny Travouillon, Mary-Anne Binnie and Liz Reed for assistance in accessing relevant collections.

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Chapter 5

Dietary diversity in Pleistocene Kangaroos of South-eastern Australia

'Some of the Pleistocene kangaroos (the Sthenurinae), acquiring massive jaws and thickly wrinkled molars, may have fed upon the coarser shrubs of the forest.'

-H. Raven & W. Gregory, 1946*

5.0. Context

This chapter investigates the diets of different kangaroo species, past and present, using Dental Microwear Texture Analysis. It considers a broad range of DMTA variables and uses an extensive modern dataset to determine which variables are best suited for understanding kangaroo diets. The diets of different kangaroo species represented in a middle Pleistocene assemblage from southeastern Australia are inferred, leading to considerations of dietary adaptations of kangaroos.

5.0.1. Statement of Authorship

Sam Arman designed the study, collected, and analysed the data used in this chapter, and wrote the manuscript.

Gavin Prideaux helped guide the focus and scale of the project and contributed to the discussion.

*Raven, H. C., & Gregory, W. K. (1946). Adaptive branching of the kangaroo family in relation to habitat. *American Museum Novitates*, **1309,** 1–33.

Dietary diversity in Pleistocene Kangaroos of South-eastern Australia

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5.1. Abstract

Kangaroos (family Macropodidae) are the dominant vertebrate herbivores of modern Australia, and were even more diverse and abundant during the Pleistocene. There were two main lineages: sthenurine kangaroos, which have mostly been considered dicot leaf browsers, and macropodine kangaroos, considered predominantly grazers. The reliance of sthenurines on browse has been implied by some as a principal reason for their extinction through climate change. Hypotheses regarding these adaptations though are based almost entirely on morphology, rather than more direct dietary inference. Here we use Dental Microwear Texture Analysis to characterise diets of the diverse middle Pleistocene macropodid fauna sampled in the Victoria Fossil Cave assemblage, alongside a large extant baseline, to consider diets of Pleistocene kangaroos. We find that sthenurine species were predominantly browsers, but that some were strict browsers while others had mixed diets. Pleistocene macropodines also evince more mixed feeding microwear signatures than their modern counterparts, which may reflect significant dietary plasticity in response to environmental change. It seems unlikely that broad changes in climate or flora can alone account for the extinction of all species of sthenurine kangaroos given the dietary breadth of the group and the range of habitats and regions that they occupied.

5.2. Introduction

Kangaroos (family Macropodidae) are the principal endemic vertebrate herbivores of Australia and New Guinea. In addition to approximately 60 living species, many other predominantly larger species occupied Australia during the Pleistocene, but went extinct around 50,000 years ago (Prideaux 2004). This included all species in the subfamily Sthenurinae, most of which were distinctly 'short-faced', and had robust builds and low-crowned, crenulated molars (Prideaux 2004). In contrast, the subfamily Macropodinae fared comparably well through this period, losing fewer species, although notably all species of *Protemnodon* (Dawson 2004). Observations on skeletal morphology have consistently been used to argue that sthenurines were primarily browsers, leaving the grazing niches for macropodines (e.g., Raven and Gregory 1946, Bartholomai 1963, Sanson 1991, Wells and Tedford 1995, Prideaux 2004). In turn, some have argued that sthenurines and other large species, such as the 2.5-tonne quadrupedal marsupial *Diprotodon optatum* and the flightless bird *Genyornis newtoni*, succumbed to increased aridity in the late Pleistocene due to their reliance on browse, which purportedly became diminished (e.g., Dawson 2006, Wroe and Field 2006, DeSantis *et al.* 2017). Others have suggested that human activities were, directly or indirectly, the principal 'megafaunal' extinction drivers (e.g., Roberts *et al.* 2001, Miller *et al.* 2005, Johnson *et al.* 2016a, Saltré et al. 2016).

Living macropodids fill a diverse range of dietary niches from fungivores (primarily potoroines), through browsers to grazers, but most species are mixed feeders, which consume varying proportions of dicot leaves and grasses (Arman and Prideaux 2015). Fungivores distribute hypogeal fungi symbiotic with trees, and grazers stimulate new grass growth, so both dietary guilds play important roles in ecosystem maintenance (Lunt 1991, Vernes and Trappe 2007, Prins and Fritz 2008). However, extant browsing macropodids are rare, so their effects on ecosystems are limited compared with those of equivalent eutherian browsers on

other continents, where large browsers strongly influence forest openness through their feeding and movement, and consume fruit which helps disperse seeds (e.g., McNaughton and Georgiadis 1986, Johnson 2009, Bakker *et al.* 2016). For example, in North America browsing by moose and white-tailed deer of hardwoods and the soft needled balsam fir, but not the hard needled white spruce has led to widespread 'spruce parkland' (Bakker *et al.* 2016). Indeed, following the extinction of many large Australian herbivores in the late Pleistocene, large-scale vegetation changes occurred that do not correlate with climatic shifts (e.g., Johnson 2009, dos Santos *et al.* 2013, Bakker *et al.* 2016).

Considering ecosystem functions for Pleistocene kangaroos based primarily on inferences of diet from craniodental morphology alone is problematic, because morphology is often adapted to the toughest elements of an animal's diet rather than what it most commonly eats or prefers to eat (Ungar *et al.* 2008, Constantino and Wright 2009, Ungar 2010). This is because adaptation allows species to consume a range of foods, and those which are most difficult to process require the most substantive changes in morphology (Constantino and Wright 2009). Because such foods are typically utilised as a last-resort during droughts or other resource-limiting events, these adaptations are strongly selected for (Constantino and Wright 2009, Ungar 2010). Indeed even among living kangaroos, dietary inference based on morphology differs substantially from those based on actual foods consumed (Sanson 1978, Sanson 1989, Arman and Prideaux 2015). Palaeodietary inferences of Pleistocene kangaroos also vary. Alternatives to grazing across all macropodines suggest that some, particularly species of *Protemnodon*, might have been browsers or mixed feeders (Sanson 1978, Johnson and Prideaux 2004, Butler *et al.* 2014). Within the Sthenurinae, a mixed-feeding or grazing diet has been suggested for *Sthenurus tindalei* and *Procoptodon goliah* (Sanson 1978, 1991, Wells and Tedford 1995, Prideaux 2004). Thus, resolving the diets of Pleistocene kangaroos

has the potential to help explain changes in flora and the wholesale reorganisation of Australian communities following the upheavals of the late Pleistocene extinctions.

Direct evidence of diet can be inferred through Dental Microwear Texture Analysis (DMTA), which is used to investigate the physico-chemical impacts of food, phytoliths and grit on tooth enamel during mastication (e.g., Ungar *et al.* 2003, Scott *et al.* 2006, Calandra and Merceron 2016). DMTA applies algorithmic quantification using scale-sensitive fractal analysis (SSFA) (Scott *et al.* 2006) or ISO standard surface texture analysis (STA) (Kaiser and Brinkmann 2006) to 3D surfaces scanned by confocal profilers (Ungar *et al.* 2003), and represents the culmination of several decades of refinements (Calandra and Merceron 2016). Only two studies of macropodid microwear have been published to date (Prideaux *et al.* 2009, DeSantis *et al.* 2017). The first featured comparisons between the extinct *Procoptodon goliah*, the grazer *Macropus giganteus* and "browser" *Wallabia bicolor*, though the latter is, in fact, a mixed feeder (e.g., Hollis *et al.* 1986, Di Stefano & Newell 2008, Arman and Prideaux 2015). DeSantis *et al.* (2017) considered four modern species, compared against the Pleistocene genera *Macropus, Sthenurus* and *Protemnodon*, but it is unclear which actual species of these genera were sampled.

The main aim of this study is to characterise the dental microwear patterns of a representative sample of 14 modern browsing, grazing and mixed-feeding macropodid species from across Australia and New Guinea. In addition, 14 species from the middle Pleistocene deposit of the Main Fossil Chamber of Victoria Fossil Cave (VFC-FC), Naracoorte World Heritage Area, southeastern South Australia (figure 5.1), were sampled to investigate diets of extinct taxa, and understand dietary change over time.

5.3. Methods

5.3.1. Modern samples

Modern specimens used in this study are housed in the: Australian Museum, Sydney (prefix AM M), Museums and Art Galleries of the Northern Territory: Museum of Central Australia, Alice Springs (CAM U); Museum Victoria, Melbourne (MV C, DTC); Queensland Museum, Brisbane (QM A, J, JM), South Australian Museum, Adelaide (SAM M); Western Australian Museum, Perth (WAM M); and the American Museum of Natural History, New York (AMNH). Palaeontological specimens used, including both extinct and extant taxa, are housed in the South Australian Museum, Adelaide (SAM P, FU). Fourteen extant species were sampled to represent a broad dietary spectrum (table 5.1). Although low numbers of specimens were available for some species, these were bolstered by increased sampling within individual specimens (chapter four).

5.3.2. Fossil samples

VFC-FC represents the best-known deposit in the Naracoorte World Heritage Area and preserves the richest and most diverse assemblage of Middle Pleistocene vertebrates in Australia (Wells et al. 1984, Prideaux 2007). A large sample (> 2,000) of tooth-bearing specimens of macropodids representing 25 species has been recovered from the site. The deposit consists of at least eight superposed units (Reed 2003), with a minimum age of 213,000 years and a potential maximum age of 479,000 years (Ayliffe et al. 1998, Moriarty *et al*. 2000, Grün et al. 2001), although the stratigraphy and chronology of the site remains to be assessed in detail. For the purposes of this paper, we treat the assemblage as a single time-averaged sample. Fourteen macropodid species represented in the VFC-FC deposit (Reed and Bourne 2000, 2009) were sampled (table 5.1). No potoroines were included because DMTA for fungivores has yet to be thoroughly investigated.

5.3.3. Data acquisition

Specimens were cleaned and cast using standard procedures (Scott *et al.* 2006), which have recently been shown to have high fidelity in replicating microwear surfaces (Goodall *et al.* 2015). Specimen casts were scanned using two confocal profilers; a Sensofar Plμ "Connie" at the University of Arkansas, and a Sensofar Plμ NEOX "Bruce" at Flinders University. Median values of the four 102 x 139 μm^2 subscans taken on Connie were used to allow comparison with the larger 242 x 181 μm^2 scans from Bruce, following Arman *et al.* (2016). To minimise differences between profilers, the Soft Filter data processing template of Arman *et al.* (2016) was used on all scans in SensoMAP 7.1.2.7288 (Digital Surf). Data collected consisted of five SSFA and 23 STA variables, which were transformed prior to analysis to satisfy normality assumptions (see SI 6).

Table 5.1: Species sampled in this study. 'VFC-FC' refers to specimens from the main fossil chamber of Victoria Fossil Cave, southeastern Australia. Modern dietary groups from Arman & Prideaux (2015).

Group	Species	N. specimens	N. scans
VFC-FC Sthenurine	*Metasthenurus newtonae*	14	44
	Procoptodon browneorum	13	45
	Procoptodon gilli	29	137
	Procoptodon goliah	4	18
	Simosthenurus baileyi	5	13
	Simosthenurus maddocki	19	36
	Simosthenurus occidentalis	9	36
	Sthenurus andersoni	19	54
VFC-FC Macropodinae	*Lagorchestes leporides*	8	10
	Macropus giganteus	45	103
	Macropus greyi	37	144
	Macropus rufogriseus	34	73
	Protemnodon brehus	6	12
	Wallabia bicolor (palaeo)	14	33
Modern Browsers	*Dendrolagus bennettianus*	6	39
	Dendrolagus lumholtzi	30	148
	Dorcopsis atrata	4	21
	Dorcopsulus vanheurni	15	92

	Setonix brachyurus	29	120
Modern Mixed Feeders	*Lagorchestes hirsutus*	18	37
	Onychogalea fraenata	13	36
	Onychogalea unguifera	20	60
	Thylogale stigmatica	38	152
	Thylogale thetis	29	115
	Wallabia bicolor	65	252
Modern Grazers	*Macropus agilis*	35	122
	Macropus dorsalis	36	102
	Macropus robustus	130	426
	Macropus rufus	39	118

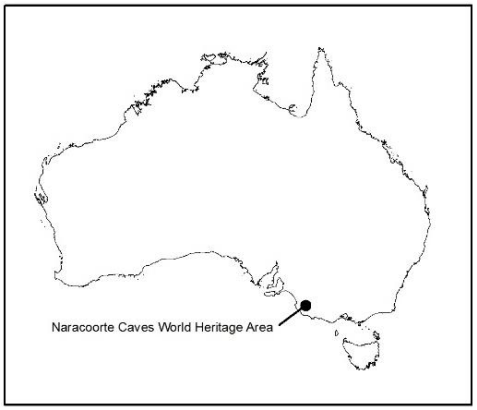


Figure 5.1: Location of the Naracoorte Caves World Heritage Area, source of fossil material used in this study.

5.3.4. Analysis

Intraspecific factors identified in chapter 4 were scored for each specimen scanned, though the *facet* reclassifications were not considered because these did not improve modelling (chapter 4). In addition, we also considered the factor *islands*, i.e., whether or not a specimen originated from an island (after Correll *et al.* 2016). Due to scanning ease, larger facets (1 and 6 for upper molars, 4 and 9 for lower molars), and wear stages 2–3 were focused upon, but not exclusively. This allowed effective modelling of differences where low numbers of specimens were available (chapter 4), and resulted in the number of scans utilised being much greater than available specimens (table 5.1).

Data were modelled using general linear mixed-models (GLMM), which allows inclusion of factors as fixed or random effects so are well suited to DMTA data (chapter 4). Multiple models were constructed and compared using Akaike's Information Criterion (AIC), which provides a score indicating how well a model predicts a given variable, and cross-validation (cvr^2) on an independent dataset (Zuur *et al.* 2009). This method has previously been applied successfully in macropodid DMTA, and have been constructed here following that methodology (chapter 4).

Initial modelling considered modern taxa with known diets along a dietary spectrum (see SI 7). Final models for all 28 DMTA variables were compared, and those that best distinguished species along dietary lines were utilised. All five SSFA variables considered were utilised: Area-scale fractal complexity (*Asfc*); considering 71 scales from 0.02–100 μm, exact-proportion Length-scale anisotropy of relief (*epLsar*) at a scale of 2 μm; Scale of maximum complexity (*Smc*); Textural fill volume (*Tfv*) at a scale of 2 μm; and Fine textural fill volume (*Ftfv*) at a scale of 0.2 μm (Scott *et al.* 2006). Five STA algorithms were also utilised: auto-correlation length (*Sal*); closed dale area (*Sda)*; maximum peak height (*Sp*); texture aspect ratio (*Str*); and material volume (*Vm*) at a ratio of 10% (Schulz *et al.* 2013).

Once response variables were chosen, the GLMM process was repeated on a larger dataset including palaeontological specimens. Diets were inferred by consensus of taxon placement across the modelled datasets. As an indicator of broad dietary differences, additional models were constructed comparing modern dietary groups and fossil subfamilies. Final models for these dietary groups and subfamilies were further analysed through ANOVA tests and Tukey's HSD post-hoc comparisons to indicate significance. Finally, the modelled estimate of each species for each variable was subjected to a Principal Components Analysis (PCA) to balance effects of species placement in the various models and visualise dietary differences. In this, the data showed multivariable normality ($p = 1$), and the PCA was constructed on a

correlation matrix as the different variables operate on different scales (Hammer & Harper 2008). All data were collected using Sfrax 1.0.11.882 (Surfract Inc.) and SensoMAP 7.1.2.7288 (Digital Surf), and analysed in R (R core team 2012) and PAST (Hammer *et al.* 2001).

5.4. Results

The ten DMTA variables considered here were chosen due to the differentiation demonstrated between extant macropodine species of known diets (figures 5.2–5.3 left). However, variability within extant dietary groups was evident, particularly for browsing taxa. Nevertheless, the ten independent variables enabled a balanced consensus to be attained for most species. DMTA data were modelled using GLMM (chapter 4), and final models produced for each variable are featured in table 5.2. Only *microscope* featured in all final models, though *ecoregion* and *facet* were featured in final models for most variables. Photosimulations of filtered, edited scans demonstrative of dietary groups are represented in figure 5.5.

Table 5.2: Final models developed through the GLMM process. SSFA: scale sensitive fractal analysis variables, STA: surface texture analysis variables. 'Trans' = transformation used. Power transformations determined by box cox log-likelihood plots (see SI 6), 'log' refers to natural logarithm.

	Variable	Trans.	Model
SSFA	*Asfc*	^0.15	Species + (1|Specimen) + Microscope + Ecoregion + Island + Facet
	epLsar	^0.63	Species + (1|Specimen) + Facet + Element + Island + Tooth + Microscope
	Smc	rank	Species + (1|Specimen) + Microscope + Facet + Coarse Wear + Island + Ecoregion
	Tfv	rank	Species + (1|Specimen) + Microscope + Ecoregion
	Ftfv	rank	Species + (1|Specimen) + Microscope + Ecoregion
STA	*Sal*	^1.25	Species + (1|Specimen) + Microscope + Coarse Wear + Facet + Element + Island
	Sda	rank	Species + (1|Specimen) + Microscope + Ecoregion + Facet + Coarse Wear
	Sp	log	Species + (1|Specimen) + Microscope + Ecoregion + Coarse Wear + Island
	Std	rank	Species + (1|Specimen) + Facet + Microscope + Coarse Wear
	Vm	log	Species + (1|Specimen) + Microscope + Coarse Wear + Ecoregion + Island

A broad dietary spectrum exists across the entire dataset, with modelled DMTA data suggesting that both sthenurine and macropodine species from VFC-FC have diets dominated by browse (figures 5.2–5.3 centre). The browse signature is strongest in sthenurine kangaroos, with VFC-FC macropodines overlapping with extant browsers and mixed feeders in modelled data for many variables. ANOVA tests were significant ($P < 0.05$) for each factor in every model *Smc* and *Sal* where the dietary/subfamily variable itself was not significant. This may suggest that for these variables, differences between these broad dietary groups cannot be detected. Results of Tukey's HSD tests between dietary groups varied between measures (table 5.3). Differences between modern browsing and grazing taxa were significant in *Tfv* and *Sda.* Modern mixed feeders were also differentiated from grazers in *epLsar* and *Sda*, but not between modern mixed feeders and browsers (table 3). While presuming dietary similarity across members of the same subfamily, fossil macropodine kangaroos differed from modern mixed feedreds in *Sp*, *Sda*, and *Std*, the latter of which also demonstrated a significant difference between fossil macropodine and modern mixed-feeding

kangaroos. Fossil sthenurine kangaroos were significantly different from modern grazing species in *epLsar, Sp, Sda, Std* and *Vm,* as well as mixed feeders in *Sp, Std* and *Vm*. No significant differences were found between fossil macropodines and sthenurines for any measure (table 5.3).

Modern DMTA data (figures 5.2–5.3 right) encapsulate a more limited dietary spectrum than when the VFC-FC data are included. Sthenurine species commonly exhibit modelled DMTA values beyond the scale seen in modern specimens. Tukey's post-hoc comparisons demonstrate where individual species differ from that of their dietary group (modern specimens) or subfamily (fossil specimens). For example, the modern grazer *Macropus rufus* demonstrated significant differences to many taxa, including other grazers in numerous algorithms. In contrast, *Simosthenurus baileyi* could not be differentiated from any taxa for any DMTA variable, while most sthenurine kangaroos have significantly different DMTA textures to numerous modern and extinct taxa. Moreover, *Si. maddocki* differs from many other sthenurine taxa. Full results of the ANOVA between-species Tukey's HSD post-hoc comparisons can be found in SI 8.

Different DMTA variables also showed some differences in which elements of diet they differentiate. Other particular subsets of species were seen in *Smc,* where significant interspecific differences were found primarily in *Dendrolagus bennettianus, D. lumholtzi*, and *Lagorchestes hirsutus* compared against other species, perhaps due to effects of *microscope* as these taxa were among the most heavily sampled on 'Connie'.

The PCA plot of the modelled GLMM outputs demonstrate the dietary spectrum evinced in the ten DMTA variables (figure 5.4). Component 1 represents 69%, and component 2 represents 13% of variance found. Clear differentiation of diets is evident in component 1, with grazers left and browsers right. Modern dietary groups are almost non-overlapping, with

the exception of the browsing *Dendrolagus lumholtzi*, which falls within the grazing hull. Several of the Pleistocene species, both macropodine and sthenurine, group around or beyond modern species at the browser end of the spectrum.

Algorithms			Modern Groups			VFC-FC macropodines			VFC-FC sthenurines			
		Diet	Grazer-Browser	Mixed-Browser	Mixed-Grazer	macro-Browser	macro-Mixed	macro-Grazer	sthen-Browser	sthen-Mixed	sthen-Grazer	sthen-macro
SSFA	*Asfc*	**<0.001**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.106	0.087	0.393
	epLsar	**<0.001**	0.226	1.000	**<0.001**	1.000	0.505	1.000	1.000	1.000	**0.037**	1.000
	Smc	0.060	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Tfv	**<0.001**	**0.016**	0.068	1.000	1.000	1.000	0.179	1.000	0.459	0.054	1.000
	Ftfv	0.003	0.062	0.126	1.000	1.000	1.000	0.491	1.000	0.323	0.067	1.000
STA	*Sp*	**<0.001**	1.000	1.000	0.695	1.000	0.695	**0.020**	0.695	**0.021**	**<0.001**	1.000
	Sda	**<0.001**	**0.006**	1.000	**<0.001**	1.000	0.065	**<0.001**	1.000	1.000	**<0.001**	0.826
	Sal	0.454	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Std	**<0.001**	0.154	1.000	0.364	0.464	**0.042**	**<0.001**	0.227	**0.007**	**<0.001**	1.000
	Vm	**<0.001**	1.000	1.000	0.815	0.942	0.815	0.080	0.195	**0.013**	**<0.001**	0.815

Table 5.3: ANOVA results for the dietary group models ('Diet'), and Tukey's HSD between dietary groups. Significant differences ($P < 0.05$) in bold. Dietary groups refer to modern specimens; 'macro' = VFC-FC macropodines; 'sthen' = VFC-FC sthenurines.

Figure 5.2 (page 167): Modelled mean and 95% confidence intervals for modelled Scale-Sensitive Fractal Analysis variables. *Thylogale stigmatica* used as dummy level for all models. a) Area-Scale Fractal complexity (*Asfc*); b) exact-proportion Length-Scale Anisotropy of Relief (*epLsar*); c) Scale of maximum complexity (*Smc*); d) Textural fill volume (*Tfv*); and d) Fine textural fill volume (*Ftfv*). Left shows species with known diets, ordered with browsers left and grazers right. Centre is modelled data for dietary groups (modern specimens) and subfamily (palaeontological specimens) ordered by mean modelled data following trends seen in modern species. Right is modelled data for all species,

ordered by mean modelled data following trends seen in modern species. Models used for final analysis can be found in table 5.1, and for extant species in SI 7. All data transformed prior to analysis to satisfy normality assumptions see SI 6. Red = browsers, purple = mixed feeders, blue = grazers, black = palaeontological specimens. Squares = sthenurine and circles = macropodine species.

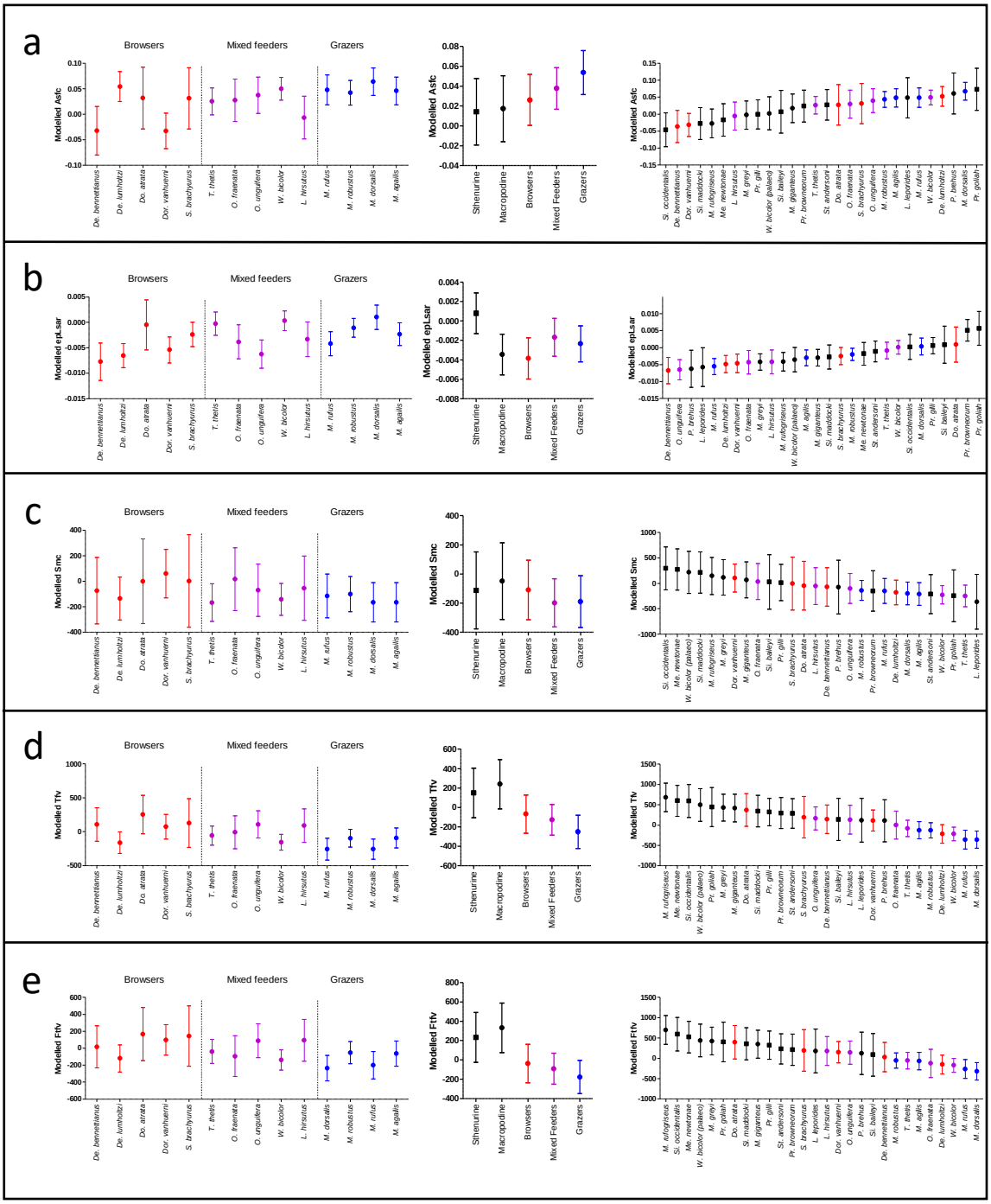
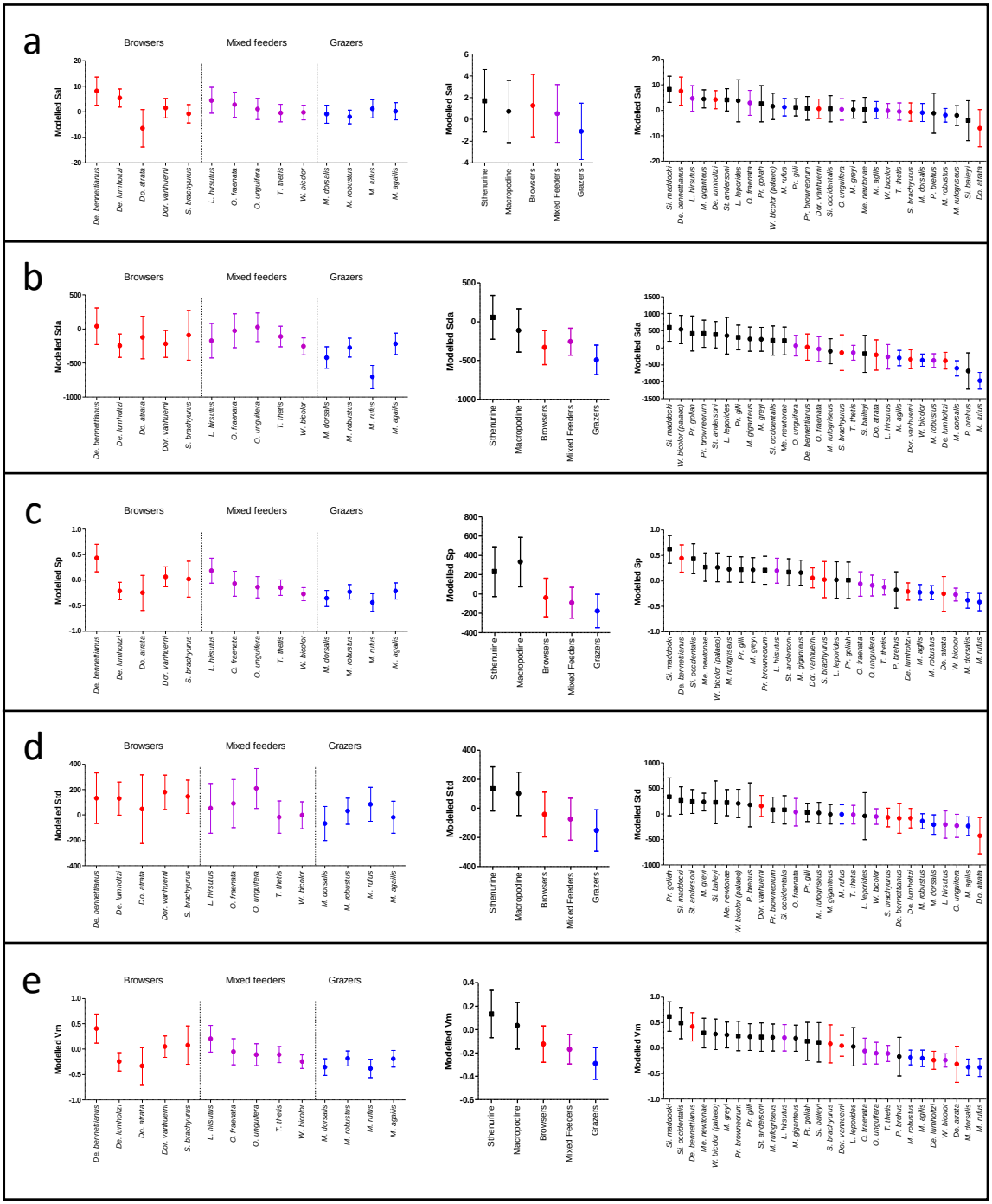


Figure 5.3 (page 169): Modelled mean and 95% confidence intervals for modelled Surface Texture Analysis variables. *Thylogale stigmatica* used as dummy level for all models. a) Auto-correlation length (*Sal*); b) closed dale area (*Sda*); c) maximum peak height (*Sp*); d) texture aspect ratio (*Str*); and e) material volume (*Vm*). Left shows species with known diets, ordered with browsers left and grazers right. Centre is modelled data for all species, grouped by dietary groups ordered by mean modelled data following trends seen in modern species. Right is modelled data for all species, grouped by dietary groups ordered by mean modelled data following trends seen in modern species. Models used for final analyses can be found in table 5.1, and for extant species in SI 7. All data transformed prior to analysis to satisfy normality assumptions see SI 6. Red = browsers, purple = mixed feeders, blue = grazers, black = palaeontological specimens. Squares = sthenurine and circles = macropodine species.


a
Browsers
Mixed feeders
Grazers
Modelled Sal
De. bennettianus
De. lumholtzi
Do. atrata
Dor. vanhuerni
S. brachyurus
L. hirsutus
O. fraenata
O. unguifera
T. thetis
W. bicolor
M. dorsalis
M. robustus
M. rufus
M. agilis
Sthenurine
Macropodine
Browsers
Mixed Feeders
Grazers
b
Modelled Sda
Mixed Feeders
c
Modelled Sp
d
Modelled Std
e
Modelled Vm

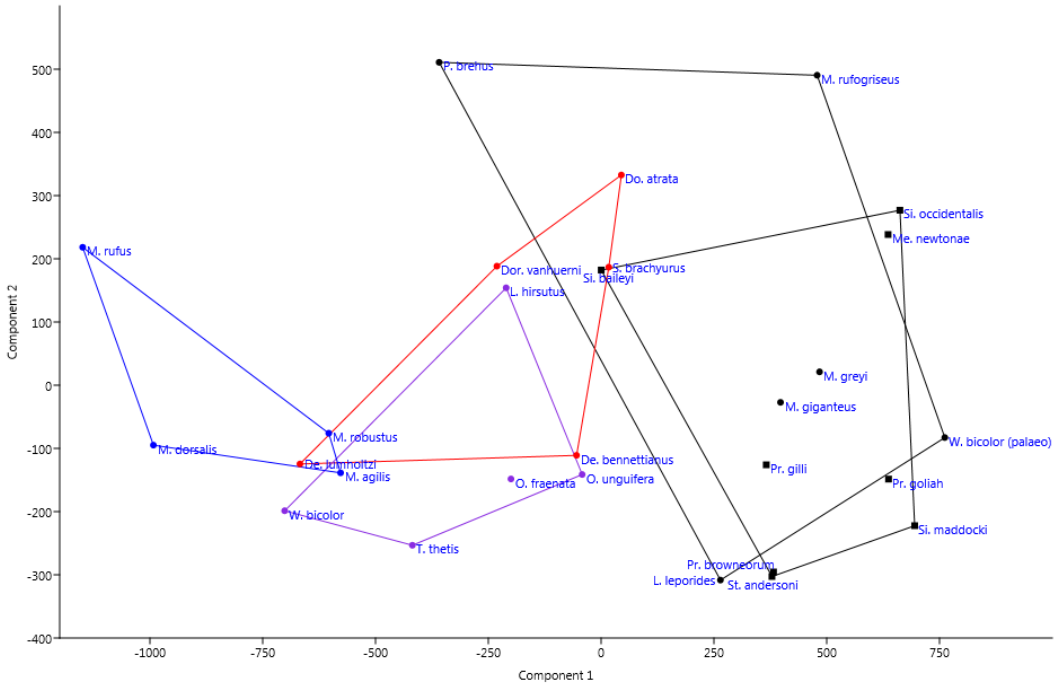


Figure 5.4: Principal Components analysis of mean and standard deviation of all final modelled DMTA data. Filled circles are modern species estimates; red = browsers, purple = mixed feeders, blue = grazers. Black squares = sthenurine palaeontological species, black triangles = macropodine palaeontological species.

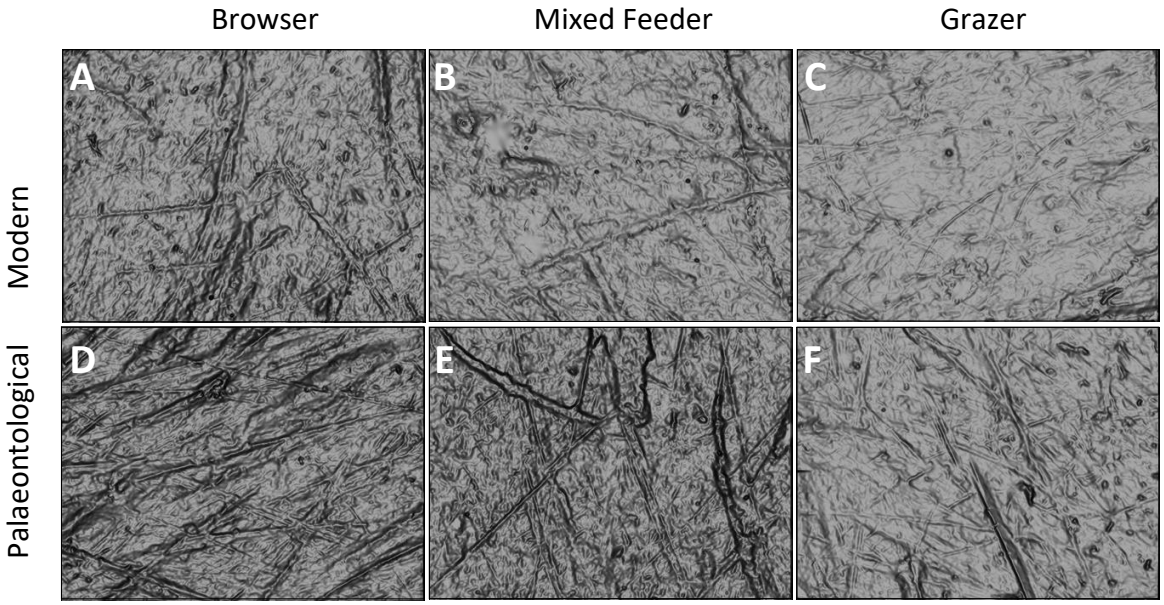


Figure 5.5: Photosimulations of scans indicative of dietary groups. All scans filtered using the 'soft filter' of (Arman *et al.* 2016) and edited to remove remaining measurement noise 'spikes'. All scans 242 x 181 μm^2. A) *Dendrolagus lumholtzi* AMNH 65249 M2 facet 9; B) *Onychogalea unguifera* QM JM 16474 M2 facet 8; C) *Macropus robustus* QM J 10736 M2 facet 9; D) *Procoptodon browneorum* SAM P28677 M2 facet 6; E) *Macropus rufogriseus* SAM P28763 M2 facet 6; F) *Protemnodon brehus* FU 0254 M1 facet 9.

5.5. Discussion

Broad differences between dietary groups demonstrate that DMTA is applicable to macropodids, through the application of a multifaceted statistical approach that incorporates interspecific variability into modelled data (chapter 4). Dietary differences visualised in the PCA constructed on modelled outputs indicate significant differences between species. Rather than dissecting full results for each species in each analysis of each variable (for which see SI 9 and summary in table 5.4), the discussion here focuses on broader scale dietary patterns.

Sthenurine kangaroos overwhelmingly exhibit DMTA data consistent with browsing, although clear differences exist between taxa. A mixed-feeding diet was inferred for *Si.*

baileyi, while mixed-feeding and browsing in different DMTA measures was evident in *Procoptodon browneorum*, *Pr. gilli* and *Sthenurus andersoni*. *Simosthenurus maddocki* may have been frugivorous in light of DMTA data being consistent with hard-object feeding in other groups. This is particularly evident in STA variables (Schulz *et al.* 2010), for which *Si. maddocki* had the highest values of any taxa in four of the five variables measured. Frugivory has previously been proposed for this species based on craniodental morphology (Prideaux 2004). No dietary consensus could be reached for *Procoptodon goliah* due to conflicting results in different variables, likely as a result of low sample size.

Microwear data for the VFC-FC macropodine species differ from those of their modern counterparts, revealing that they consumed relatively more browse. *Wallabia bicolor* demonstrates this particularly clearly as the only taxon represented in both modern and Pleistocene datasets. Marked differences between the two may indicate dietary change over time, with modern *W. bicolor* consuming more grasses at present than during the middle Pleistocene. Indeed, a browsing diet for this species accords well with its morphology (Sanson 1980). It is interesting to note that in modelling, the recently-extinct *Macropus greyi* was among the VFC-FC species with the least-variable diet, perhaps related to observations of *M. greyi* returning to preferred microhabitats (Robinson and Young 1983). The extinct *Protemnodon brehus* exhibits a grazing signature in DMTA data, which is not consistent with previous palaeodietary indices. Stable isotopes suggest a C_3 diet consistent with browsing (Montanari *et al.* 2013), as does thin enamel (Couzens 2016), while mesowear suggests mixed feeding (Butler *et al.* 2014). This may indicate that species of *Protemnodon* specialised on an abrasive C_3 resource, such as forbs, which is further supported by the lack of a molar mid-link (cristid obliqua) found in other macropodine grazers to slice tougher grasses (Prideaux and Warburton 2010).

Table 5.4: Diet as inferred from DMTA data. See SI 9 for further details. DMTA diet was inferred through a consideration of each species across the multiple models, ANOVA tests and the PCA.

Group	Species	DMTA Diet
VFC-FC Sthenurine	*Me. newtonae*	Browser
	Pr. browneorum	Browser–Mixed Feeder
	Pr. gilli	Browser–Mixed Feeder
	Pr. goliah	Unclear
	Si. baileyi	Mixed Feeder
	Si. maddocki	Browser–Frugivore
	Si. occidentalis	Browser
	St. andersoni	Browser–Mixed Feeder
VFC-FC Macropodinae	*L. leporides*	Mixed Feeder
	M. giganteus	Mixed Feeder
	M. greyi	Browser–Mixed Feeder
	M. rufogriseus	Browser–Mixed Feeder
	P. brehus	Grazer
	W. bicolor (palaeo)	Browser–Mixed Feeder

Most modern species exhibited DMTA data consistent with known diets. Some modern browsers did present confounding results, though these are likely related to issues regarding sample size and profilers used (see below). With consumption of both browse and graze in mixed-feeding species, it may have been expected that variability would be greater for mixed feeders than grazers or browsers. However, most mixed feeders were less variable than modern browsers, but not grazers. This may be the product of GLMM, which enables intraspecific variability to be incorporated into species estimates (Zuur et al. 2009). Grazers were the easiest group to distinguish in modern analyses, showing less variability within or between species in modelled data.

5.5.1. Comparisons to previous work

The first application of DMTA to kangaroos was the multi-method work of Prideaux *et al.* (2009). Although scant on methodological details, this argued for a browsing diet for *Procoptodon goliah* due to similarity of DMTA data to the presumed browser *Wallabia*

bicolor (Prideaux *et al.* 2009). Here we show that modern *W. bicolor* has microwear consistent with its mixed feeding diet (e.g., Hollis *et al.* 1986, Di Stefano & Newell 2008, Arman and Prideaux 2015). Regarding *Pr. goliah* however, the contradictory dietary signal presented here suggests that the diet of this largest of kangaroos is still unclear, particularly given its unique masticatory apparatus and stable-isotope data indicating a C_4-based diet (Prideaux *et al.* 2009). DeSantis *et al.* (2017) considers a range of macropodids from a Pleistocene site at Cuddie Springs in eastern Australia. Although it is unclear which species were sampled, all genera analysed (*Sthenurus, Macropus* and *Protemnodon*) were considered to have a browse diet in the Pleistocene (DeSantis *et al.* 2017). This accords somewhat with data presented here regarding *Sthenurus,* but contrasts with the grazing signature present for *Protemnodon brehus,* and the mixed-feeding / browsing signature presented here for species of *Macropus* from VFC-FC. The overall picture of a Pleistocene community of browsers (DeSantis *et al.* 2017) contrasts somewhat with data presented here, which demonstrates dietary variability between species, with mixed feeding rather than browsing being the norm. It may also be important to note that both Prideaux *et al.* (2009) and DeSantis *et al.* (2017) do not state which teeth or facets were scanned, so intraspecific effects of these or other parameters may affect data collected (chapter 4).

Stable-isotope analysis for north-eastern Australian Pliocene macropodids suggest that *Macropus* fed on a mix of C_3 and C_4 plants (Montanari *et al.* 2013), as is consistent with mixed-feeding in *Macropus* evinced here. A C_3 diet for *Protemnodon* (Montanari *et al.* 2013), contrasts with grazing DMTA data, but as discussed above, may indicate a forb-specialist diet. A C_3 diet for sthenurines may also be apparent in the floral record, which shows a short-lived increase of C_3 browse following the extinction of sthenurine kangaroos, but without correlating to any widespread changes in climate (dos Santos *et al.* 2013). A mixed-feeding diet for *M. giganteus* and *Protemnodon brehus* is indicated by mesowear

analysis (Butler *et al.* 2014). This contrasts with results here regarding *P. brehus*, but accords with the categorisation here of Pleistocene *M. giganteus* as a mixed feeder. Enamel thickness data indicates that sthenurine kangaroos are characterised by thinner enamel than macropodines (Couzens 2016), which is consistent with dietary differences inferred from our DMTA data.

Functional morphology of sthenurine kangaroos has long suggested a browsing diet (e.g., Raven and Gregory 1946, Bartholomai 1963, Wells and Tedford 1995). Although the some degree of browsing inferred here for most sthenurine species accords well overall with the morphology-based assessments, DMTA data show that *Sthenurus andersoni* and *Simosthenurus baileyi* undertook a certain amount of mixed feeding. Indeed a mixed-feeding or grazing diet for *Sthenurus* was suggested by Tedford (1966), while Prideaux (2004) goes on to suggest *Sthenurus* may be more likely to be mixed feeding than other sthenurine genera, possibly specialising on low, dusty forbs. Suggestions of a highly-specialised diet browsing on seeds, fruits or otherwise for *Si. maddocki* based on molar morphology (Prideaux 2004) also accord with data presented here. A propensity of *Metasthenurus newtonae* and *Procoptodon gilli* to browse on softer, but more abrasive vegetation has also been suggested (Prideaux 2004). Teasing apart the roles that food and grit play in microwear formation is an ongoing source of debate (e.g., Ryan 1979, Ungar *et al.* 1995, Lucas *et al.* 2013), but a browse diet is certainly supported for both these species. At a generic level, phylogenetic analysis reveals specialist browsing characters for *Metasthenurus* and *Procoptodon* (Prideaux and Warburton 2010), and morphology suggests these genera were able to reach above the head to attain high browse (Wells and Tedford 1995), again consistent with browsing DMTA data. It is poignant to note that *Me. newtonae* and *Si. occidentalis* have very similar distributions (Prideaux 2004). That they too share a common DMTA signature, especially apparent in the PCA, demonstrates a potential link between diet and distribution for these

species, possibly due to preference for a particular resource. This further supports the idea that DMTA can, for some species, be used to infer environmental conditions (e.g., Burgman *et al.* 2016, Merceron *et al.* 2016, chapter 4).

Functional morphology has also suggested a grazing diet for *Procoptodon goliah* (Sanson 1978, 1991, Wells and Tedford 1995), but as mentioned above, a dietary consensus for this species remains elusive. Craniodental morphology has been used to infer that *Pr. browneorum* was adapted for browsing on tough vegetation, twigs and fibrous leaves (Prideaux 2004), but this is only partially supported by the DMTA data, suggesting browsing alongside mixed feeding to be apparent. This may however be a case of fall-back feeding, where morphology is adapted to the toughest elements of an animal's diet (Scott *et al.* 2005, Constantino and Wright 2009). Indeed, given that most sthenurine kangaroos considered here show some degree of mixed feeding, it may be that specialised browsing interpretations of sthenurine morphology across the board have been somewhat misled due to this phenomenon. In the same regard, increased mixed-feeding by Pleistocene macropodines supports the notion that, while adapted to a high-grazing diet, they may also have broader diets where facilitated by environmental conditions (e.g., Beetham *et al.* 1987, Green *et al.* 2014, Arman & Prideaux 2015). Species of *Protemnodon* have previously been considered browsers (Sanson 1978), which contrasts with data presented here. This further suggests that the 'megafaunal' extinction event is driven by non-dietary factors (Miller *et al.* 2005, Prideaux *et al.* 2009, Johnson *et al.* 2016a).

5.5.2. Macropodoid dietary ecology

Overall, the picture presented by the diets of VFC-FC kangaroos is that of an extended dietary spectrum, with most palaeontological specimens exhibiting data beyond the scale seen in the extant baseline. In addition, increased consumption of browse by *M. giganteus*, *M. rufogriseus* and *W. bicolor* in the past may reflect dietary plasticity in response to variable

conditions, as is apparent for many modern species (Arman and Prideaux 2015). Dietary plasticity within herbivorous groups has been observed elsewhere through microwear, with fossil *Bison* showing more mixed feeding than modern specimens, which were all grazers (Rivals and Semprebon 2011). Other variability within dietary groups has been noted in primates, predominantly due to occasional hard-object feeding (Scott *et al.* 2005, Ungar *et al.* 2010). Variation across regions is more common (e.g., Merceron *et al.* 2010, Rivals *et al.* 2012, Burgman *et al.* 2016), but because *ecoregion* was incorporated into most models here, such variation is taken into account, at least for modern specimens. Microwear differences between species of *Simosthenurus* may be due to niche partitioning, as is seen in other macropodids today (e.g., Vernes 1995, Shepherd *et al.* 1997, Davis *et al.* 2008), and may be widespread where kangaroo diversity is high.

All extant browsing macropodids have a mean adult body mass of < 15 kg (Van Dyck and Strahan 2008), considerably less than the sthenurine kangaroos, which range from ~50 to 250 kg (Helgen *et al.* 2006). It certainly seems extremely unlikely that a ~118 kg *Si. occidentalis* would eat the same plants or parts of plants as a < 5 kg *Setonix brachyurus,* even if both species are browsers (Helgen *et al.* 2006, Van Dyck and Strahan 2008). This is especially true when considering that some sthenurines could evidently reach above their head to pull down branches upon which to feed (Wells and Tedford 1995). Interestingly, a number of species of shrubs and trees in Australia have anti-herbivore defences that now appear obsolete, because they are rarely fed upon by modern species (Johnson 2009). For example, many species of *Acacia* have spines in their juvenile forms, where browsing would be most detrimental to the plant, but these spines disappear in adult forms where leaves are beyond what even the tallest browsers could reach (Johnson 2006, 2009). Feeding height could play a particularly important role in distinguishing low, dusty shrubs from other browse, as suggested by Prideaux (2004) for some sthenurines, particularly given the role that grit appears to play in

microwear formation, and how this varies at different feeding heights (Ungar *et al.* 1995). Extant tree-kangaroo species (*Dendrolagus*) may be particularly important in teasing apart these contributions to microwear formation, though *Dendrolagus* samples here appear limited by sampling issues.

The dietary spectrum covered by extant macropodids has previously been seen to contrast with those of other herbivorous groups (Arman and Prideaux 2015), but such differences appear less prevalent with the inclusion of the relatively recently-extinct sthenurines. Dietary classification of extant bovids identify numerous categories not evident within macropodoids (Arman and Prideaux 2015). For example bovid mixed feeders were divided into browser–grazer intermediates and generalists (distinguished by whether they include fruit alongside grasses and browse in their diet), as well as the identification of numerous bovid frugivore species (Gagnon and Chew 2000). Dental microwear data also differentiates between these bovid dietary groups, even differentiating obligate and variable grazers in some cases (Merceron *et al.* 2004, Ungar *et al.* 2007, Scott 2012). These certainly contrast with results seen in the modern macropodoid analyses here, however, when considering VFC-FC taxa, there may be grounds to separate sthenurines into obligate (*Me. newtonae* and *Si. occidentalis*) and variable (*St. andersoni, Si. baileyi, Pr. browneorum* and *Pr. gilli*) browsing groups. In bovids, six species of *Cephalophus* (duikers) consume > 70% fruit (Gagnon and Chew 2000), and this signal was detected in DMTA (Scott 2012). Moreover mixed-feeding bovids (10 species) all consume > 20% fruits. In living kangaroos, only the fungivorous potoroines and *Hysiprymnodon* eat significant amounts of fruit, and none of these in levels approaching duikers (Arman and Prideaux 2015). DMTA of kangaroos however identified *Simosthenurus maddocki* as a frugivore. Frugivory in *Si. maddocki* and potentially other sthenurines, could be ecologically significant as dispersers of seeds in feedback loops maintaining preferred plants (Johnson 2009).

Considering fruit dispersal by sthenurines will require investigation of what fruits, seeds or other foods containing hard objects are present in Pleistocene Australia and could have formed this specialist diet. Other differences between bovids and macropodids remain. Bovid diets have been shown to correlate so well with habitat that the presence of a particular species is used to infer environmental conditions (Ungar *et al.* 2007, Scott 2012). In contrast, dietary variation across ranges of extant kangaroos was so great that *ecoregion* was utilised in the GLMM process for seven of the ten variables utilised, to incorporate differences between regions into models of inter-specific differences. However, similarity between *Si. occidentalis* and *Me. newtonae*, and low variability in *M. greyi* DMTA data may indicate habitat sensitivity for some species.

5.5.3. DMTA data and GLMM

Prior to the full analysis, this study considered a broad range of DMTA variables (see SI 7). Some variables not utilised in the final analysis were rejected because they did not reveal any differentiation of extant species with known diets, possibly because they reflect non-dietary elements of surface texture (Calandra and Merceron 2016). Numerous STA height (*Sq, Sv, Sz* and *Sa*), and volumetric (*Vmc, Vvc* and *Vvv*) measures were not included because they yielded identical results to *Sp* and *Vm*, which were considered representative of these groups of variables (see SI 7).

Data here for complexity (*Asfc*) contrast to the general consensus for this variable, where browsing taxa are typically higher in complexity than grazers (Scott 2012, Calandra and Merceron 2016), as well as a preliminary analysis of macropdid microwear (chapter 4). This most likely reflects the lack of heavily ‘pitted’ surfaces typically associated with browsing (Merceron *et al.* 2004, Scott 2012). As seen in figure 5.5, browse signatures of kangaroos are more typically thick, deep scratches, which may be harder to analytically differentiate in *Asfc* than the typical “pits versus scratches” dichotomy (Calandra and Merceron 2016). However,

microwear of *Mammut americanum* do exhibit 'hypercoarse' scratches more akin to those seen in macropodine browsers (Rivals *et al.* 2012). Given that proboscideans and macropodids are both lophodont and have teeth that progress anteriorly in the jaws during their life (Lentle 2003, Ungar 2010), molar morphology may be a key driver of differences in microwear textures between such forms and other herbivorous groups, such as ungulates and primates. Other SSFA variables performed similarly to other dietary groups, such as high *epLsar* grazers, and high *Tfv* browsers (Scott 2012). Moreover, high values seen here for STA variables are consistent with increased hard-object feeding in browsing taxa (Schulz *et al.* 2010).

Problematic results seen for the modern browser *Dorcopsis atrata* may be related to a low sample size (n=4, 21 scans), in contrast to *Dorcopsulus vanheurni* (n=15, 92 scans), which had a clearer browse signature. Low sample size may also play a role in the conflicting dietary consensus for the sthenurine *Procoptodon goliah* (n=4, 18 scans). Difficulties in attaining a consensus across DMTA data for the two species of *Dendrolagus* is likely also due to sample size as well as profilers used. These two taxa were the most heavily sampled on 'Connie', and this effect may have transcended employment of the soft filter template (Arman *et al.* 2016) and modelling *microscope* effects in GLMM. Low sampling of other species and diets on 'Connie' may also have limited the degree to which modelling could incorporate profiler effects into species estimates. These problematic data may also have

Variability within extant dietary guilds suggests that further fine-tuning of DMTA analysis is warranted. Additional variables may always be incorporated into the GLMM process, such as body size. Many species also exhibit similar DMTA data to other members of the same genus (e.g., *Macropus, Procoptodon*). This could be due to dietary similarity as well as the dental morphology that unites genera also breaking down foods in comparable ways. This could mean that tying DMTA to phylogenetic analysis could assist in refining dietary differences.

The omnipresent possibility yet to be fully realised is also to tie DMTA analyses to other palaeodietary proxies, such as stable isotopes, crown height and mesowear. Dietary inference was undertaken here using a consensus approach with the five SSFA and five MTA variables. 1With these variables being largely complementary, analysis may be further improved by incorporating these into a single balanced model.

5.6. Conclusion

Dietary and taxonomic diversity of kangaroos in Pleistocene Australia suggests that all kangaroos were well adapted to the variable climatic fluctuations characterising this period. However, comparing the diets of macropodine and sthenurine kangaroos, we find broad differences to be apparent. Many sthenurine species were specialist consumers of browse resources, even fruits, while others were more mixed feeding. Macropodine species also show increased mixed feeding in the past. These results indicate that, while browsing and grazing morphology separate macropodine and sthenurine kangaroos, these are largely evolutionary adaptations for processing particular elements of their diets: tough browse for sthenurines and strong grasses for macropodines. However, both show evidence of utilising all available foods.

5.7. Acknowledgements

Thanks to Larisa DeSantis for providing casts of the AMNH specimens of *Dorcopsulus vanheurni.* Thanks to Thomas Prowse for continual assistance with GLMM. Thanks to Sal Caporale and Sarah Livengood for assistance in scanning at the University of Arkansas. Thanks to Sandy Ingleby, Adam Yates, Karen Roberts, Heather Janetzki, David Stemmer, Kenny Travouillon and Mary-Anne Binnie for assistance in accessing relevant collections.

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Chapter 6

Discussion

'There are known knowns. These are things we know that we know. There are known unknowns. That is to say, there are things that we know we don't know. But there are also unknown unknowns. There are things we don't know we don't know.'

-Donald Rumsfeld 2002*

6.0. Context

This chapter is a broader discussion on kangaroo diets, the current state of dental microwear texture analysis in palaeodietary inference, and potential avenues for further research.

*United States Secretary of Defence Donald Rumsfeld, Press Conference, February 12, 2002.

Discussion

Data presented in this thesis offer a glimpse into the diets of kangaroos (superfamily Macropodoidea). Although largely supporting existing interpretations of diet, findings prompt further questions about the relationship between adaptation, diet and the broader ecological community. However, these data also raise new and reiterate many old questions about microwear formation and methodology, which have serious implications for how we undertake DMTA and interpret results.

6.1. Kangaroo Ecology

Extracting data from published literature yielded reliable proportional dietary data for 19 species, and categorical data for 37 species, out of the ~70 living macropodoid species (chapter 2). In other words, there are no published dietary data for just under half of the living species, including all representatives of *Dorcopsis* and *Dorcopsulus*. Diet is one of the most central elements of the biology of all animal species, and a key determinant of behaviour and survival. This dearth of information may be critical for the effective management of some living kangaroos, particularly those that are currently endangered or vulnerable. Indeed, the natural dietary variability observed in several species through space (chapter 2), and time (chapter 5), may provide insight into the adaptability of species and their potential suitability for relocation where current habitats are threatened.

Widespread mixed feeding in modern kangaroo species (chapter 2), is likely an adaptation to the influence of climatic variability on vegetation patterns, which is reinforced by the middle Pleistocene evidence from southeastern Australia (chapter 5). By contrast, mixed feeding is an uncommon strategy in artiodactyls, such as bovids, which tend to specialise in browsing or grazing guilds (Gagnon and Chew 2000). Phylogenetic analyses suggest that most crown-group kangaroo genera diversified by the late Miocene (Prideaux and Warburton 2010), as

Australia became increasingly arid as it drifted north from Antarctica (Fujioka and Chappell 2010). By the Pliocene, much of Australia was semi-arid, a trend that continued into the Pleistocene, as the climatic fluctuations that characterise that period cycled between warm-wet and cool-dry periods (Fujioka and Chappell 2010). This suggests that the climate, flora and fauna were well established prior to the Pleistocene. While Pleistocene fluctuations certainly presented challenges, they by no means prevented the establishment of well-delineated browsing or grazing guilds in kangaroos. That this was not the case then requires further consideration, possibly through better understanding the dynamics of Australian climatic variability which may encourage dietary flexibility, or considering which dietary niches were occupied by other herbivores.

In contrast to the evidence from ecological and microwear data, which suggests that macropodid species are relatively evenly distributed along a dietary spectrum (chapters 2 and 5), craniodental morphology clusters taxa into distinct groups hitherto argued to correspond to different dietary categories (e.g., Sanson 1978, 1989, Prideaux and Warburton 2010). Uniting direct dietary evidence with morphology may create a scheme more fitting to the different types of mixed-feeding diets evinced here. For instance, classification may consider browse-adapted and graze-adapted mixed feeders distinctly, possibly in relation to extremes of diet, fall-back feeding, morphology or other factors.

Dietary variability in some sthenurine kangaroos present in the middle Pleistocene assemblage of the Fossil Chamber of Victoria Fossil Cave, and similarities in diet to coeval macropodine kangaroos (chapter 5), do not support wholesale sthenurine extinction through broad changes in climate or flora. This accords with previous studies which suggest that across marsupials, diet does not indicate extinction risk (Johnson and Prideaux 2004). Human impacts on “megafauna” have a well-documented global record. In Australia, the debate has been muddied somewhat due to uncertainties of dating both extinctions and human arrivals

(Johnson 2005). Recent work though continues to push back temporally the arrival and dispersal of humans across Australia (e.g., Hamm *et al.* 2016, Clarkson *et al.* 2017, Tobler *et al.* 2017), while refined dating of fossil localities (e.g., Johnson 2005, Prideaux *et al.* 2010, Jankowski *et al.* 2016) leave a substantial temporal window of overlap between humans and megafauna. With such solid circumstantial evidence, it is now a matter of refining how the interplay between human activities (e.g., landscape altering, hunting, competition) and both extrinsic (e.g., climate) and intrinsic (e.g., biological) factors led to the extinctions. And as always, increased understanding of the biology of these animals and their diets will continue to contribute to refining these causes.

Flow-on effects may also be apparent from the loss of sthenurines and other large herbivores, which would have physically opened up vegetation through diet and movement, and could also could have resulted in the loss of co-evolved plants (Johnson 2009, Bakker *et al.* 2016). In particular, fruits dispersed by herbivores often require digestion for germination (van Wieren and Bakker 2008), so loss of the apparent frugivore *Simosthenurus maddocki* (chapter 5) would have had direct consequences on plants it was utilising. Reorganisation of plant–herbivore communities can also have effects on biodiversity of other vertebrates, plants, invertebrates, and even bird communities (van Wieren and Bakker 2008). Large-scale vegetation change has been demonstrated in North America, Europe and Russia following megafaunal extinctions (Johnson 2009). In Australia, pollen cores show a marked reduction of rainforest species following the megafaunal extinction, which were replaced by sclerophyllous plants and an increase in burning (Rule *et al.* 2012). More broadly, offshore cores show a short-term increase in fuel biomass, and a 32% drop in C_3 plants following the megafaunal extinctions, likely through lack of browsing (dos Santos *et al.* 2013). Refining the relationship between herbivores and flora over the Neogene will help understand evolutionary trajectories and help manage modern Australian biota.

6.2. Refining macropodid microwear

Any single palaeontological assemblage samples only one area within the geographic range of a species, which for kangaroos, including sthenurine species, can be tens of thousands of square kilometres (Prideaux 2004, Van Dyck and Strahan 2008). This then limits the extent to which the dietary interpretation of VFC-FC specimens (chapter 5) can be inferred across species and regions. Understanding how much geographical variability is present in the Pleistocene will require these same species be sampled from elsewhere, particularly given that DMTA of living species varies by *ecoregion* (chapter 4).

Tracking microwear signals within species through individual stratigraphic sequences has the potential to reveal whether species adjust their diets over time in response to environmental change and, if so, how. One might anticipate that such temporal variation may mirror that seen between ecoregions (chapters 4–5). The middle Pleistocene assemblage analysed here represents a time-averaged sample likely spanning a minimum of one full, 100,000-year glacial–interglacial cycle (Ayliffe *et al.* 1998, Moriarty *et al.* 2000, Grün *et al.* 2001). Once the Victoria Fossil Cave deposit is better dated and the precise relationship of horizontal spits within which fossils were excavated and the stratigraphy is verified, it will be possible to further investigate DMTA data for potential dietary change through time within those species well represented through the entire section (e.g., *Macropus rufogriseus, M. giganteus, Procoptodon gilli*). Other well-stratified, well-dated deposits that may be amenable to assessment include the infill sequences in Leaena's Breath Cave, south-central Australia (Prideaux et al. 2007), and Cathedral Cave, Wellington, New South Wales (Dawson and Augee 1997). Considering variation within species through space and time will also help tease apart the degree to which microwear patterns reflect underlying flora compared to dietary preferences for each species, particularly when the dietary signature is combined with relative abundance of species present (e.g., Ungar *et al.* 2007, Burgman *et al.* 2016). This

approach could also help investigate questions regarding specialist diets, such as whether *Protemnodon brehus* is indeed a forb specialist (chapter 5), by comparing its diet to those of the broad palaeocommunity, particularly *Macropus giganteus* which is known to be a less selective 'bulk feeder' compared to other large *Macropus* species (T. Dawson pers. comm. 25/8/17). This may make *M. giganteus* also well-suited to refine floral changes over the Quaternary following ecosystem reorganisation associated with megafaunal extinction. Given extensive landscape modification following Aboriginal and European arrival in Australia (Johnson 2006) it may also be worth considering changes within macropodine species over this period, and whether the grazing signature of modern kangaroos has recently increased because of this.

Analytical protocols require continual reappraisal to determine how appropriate they are for the data used and the phenomena those data describe. The use here of general linear mixed modelling (GLMM) to describe microwear data is unique; past DMTA studies have typically employed ANOVA or the non-parametric equivalent, Kruskal–Wallis tests to identify differences between species or dietary groups (e.g., Scott *et al.* 2005, Prideaux *et al.* 2009, Purnell *et al.* 2013). Using GLMM, enables intraspecific variability to be incorporated into modelled estimates of differences between species. Utilising large sample sizes and seeking consensus across multiple variables and statistical techniques (chapter 5) provides a nuanced but well-supported understanding of dietary differences in DMTA data.

The use here of mathematical transformations to normalise data also contrasts with the standard approach of rank transforming DMTA data (e.g., Scott *et al.* 2005, Prideaux *et al.* 2009, Calandra and Merceron 2016). Rank transformation is typically considered a 'last-resort' approach by statisticians, because rank-transformed data do not translate easily to input data, and are particularly difficult to translate to secondary terms in analysis (Saltelli and Sobol 1995), such as those employed in the GLMM models (chapters 4–5). Rank

transformation, though, has become ingrained in DMTA, largely because of its simplicity, especially given the difficulty of attaining normality for many DMTA variables (Calandra and Merceron 2016). However, box-cox plots of log-likelihood can be extremely useful in finding suitable mathematical transformations of DMTA variables (Chapters 4–5). The utility of such transformations is that they retain a closer resemblance to the raw data, and so preserve more of the spread of the data than rank transformations (Saltelli and Sobol 1995). Box-cox is not a magic bullet though, and rank transformations were still used in half of the DMTA measures used (chapter 5). However, by utilising more sophisticated statistical techniques, DMTA researchers can do better at extracting meaning from their microwear data than by using rank transformation alone.

The introduction of filters to minimise differences between profilers (chapter 3) may also have played a role in the lowered variation seen between species (chapter 5). The 'Soft Filter' used extensively here applies a range of processes to surfaces, such as filtering patterns at a particular scale, and removing the highest and lowest 0.1% of scans to eliminate measurement noise (chapter 3). Systematic removal of information from scanned surfaces has the potential to remove features that are dietary, rather than associated measurement noise, particularly given that removed areas are filled using a smoothing algorithm that will be similar for all scanned surfaces (chapter 3). As such, the use of this template may result in the filtered surfaces being more similar than unfiltered surfaces would be (chapter 3), and so could theoretically reduce differences between dietary groups.

Systematic differences between profilers plagued the analysis, with the factor *microscope* utilised in all final GLMM models (chapter 5), despite employment of the 'Soft Filter' (chapter 3). Moreover, final ANOVA models, which are less able to incorporate factor variation into species estimates, were focused on species heavily sampled on one profiler in some variables (chapter 5). Future work may wish to address this concern by only

incorporating data collected on a single profiler, though this would greatly limit comparability between laboratories, undermining one of the major purported advantages of the method (e.g., Ungar *et al.* 2003, Scott *et al.* 2005, Calandra and Merceron 2016). The steps contained in the 'Soft Filter' template were aimed at removing 'spikes' associated with measurement noise (Calandra and Merceron 2016). Observationally, these steps were successful, with fewer 'spikes' seen in filtered scans, which greatly simplified the time-consuming artefact removal process, while resulting in surfaces that still resemble those of the original scans. Ultimately, the utility of this technique will be determined by whether the template is utilised by workers elsewhere, and what effect this has on DMTA data for groups where dietary differentiation is better understood (e.g., primates, bovids).

One manifest indication of challenges that lie ahead is provided by a case study of koala diets using DMTA (Hedberg and DeSantis 2017). Even though one of the authors had hitherto co-authored works identifying disparities in comparability of DMTA results from different profilers (Ungar *et al.* 2014), and a suggested means to temper them (Arman *et al.* 2016), the potential for confounding effects was dismissed, even though dietary differences highlighted corresponded with the different profilers used (Hedberg and DeSantis 2017). Moreover, the study cited Arman *et al.* (2016) as evidence of the lack of differences between profilers, even though the main observation of that paper was that such differences did exist.

Interpreting macropodid microwear offers some new challenges to DMTA, through confounding data seen in some variables (chapter 5). One avenue to better resolve differences in DMTA data may be to more fully capitalise on the scale-sensitive nature of *epLsar* (anisotropy). At present, anisotropy utilises cross-section lengths at different orientations at a single scale (Scott *et al.* 2006). This has been sufficient in many groups for distinguishing heavily-scratched grazer teeth from less-scratched browser teeth. In kangaroos, where both browsers and grazers exhibit scratched teeth, but are differentiated by the coarseness of

scratches, analysis of *epLsar* across different scales of measurement (as is possible with existing software) may prove enlightening.

6.3. Future research

That dental microwear patterns reflect diet has only been recognised for half a century, and DMTA has existed for less than two decades (Calandra and Merceron 2016). Not surprisingly, methodologies are still being developed and refined (figure 6.1). Some developments are responsive to observations, such as noted differences between profilers (Ungar *et al.* 2014), which led to the filters developed in chapter 3 and utilised in chapters 4–5. On the other hand, researchers undertaking DMTA must import their understanding of the study organisms' biology into both data collection and analysis. Molar progression in kangaroos moves both upper and lower teeth anteriorly during an animal's life (Lentle *et al.* 2003). As such, a third molar is the posterior-most tooth in young kangaroos, but can be the anterior-most tooth in older kangaroos as M1 and M2 are lost (Kirkpatrick 1964). This means sampling the same tooth position (e.g., M2) across individuals, as is standard microwear practice, does not necessarily result in an equivalent tooth position. Consideration of these effects led to incorporation of intraspecific effects into species' dietary estimates (chapter 4). Importantly, the GLMM process resulted in final models in the full dataset (chapter 5), largely focusing on factors other than *tooth* and *wear*, which it was initially intended to incorporate. This demonstrates that while methodological studies may set out to address a single problem, they also can result in broader applications. In all cases, though, efforts should be made to disseminate methodological advances and incorporate them into DMTA practices.

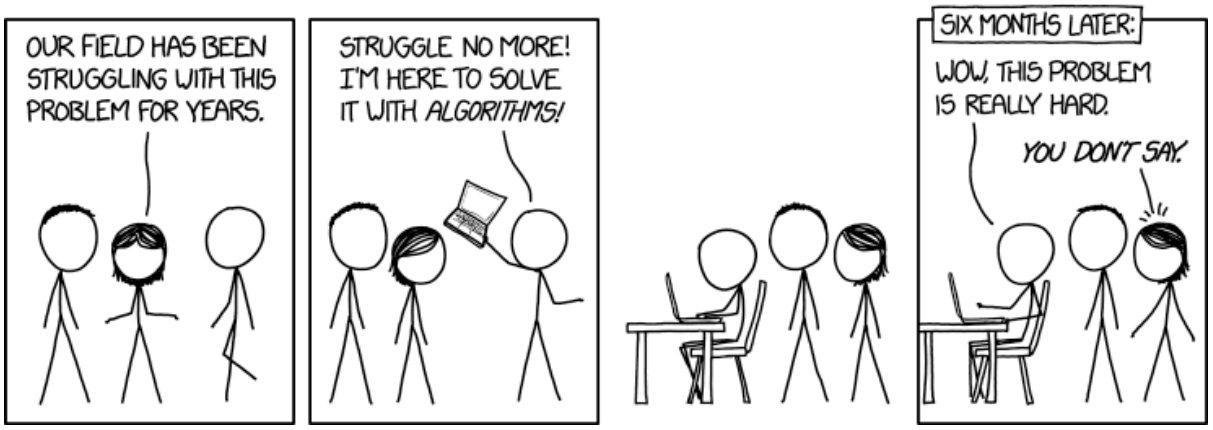

Figure 6.1: Methodological advances in DMTA. Source: 'Here to help' <https://xkcd.com/1831/>

There are a number of clear fronts for future microwear research on living kangaroos. As more is known of living species diets, as well as their environment, living species can help refine how dietary differences are exhibited in microwear data (chapter 5). Further fine-tuning what can be inferred from modern specimens will depend on what information can be gleaned from museum specimens. Given that many museum specimens contain both geographic and temporal information, these lines may be considered through further analysis, such as the factor *islands,* added opportunistically in chapter 5, which showed considerable utility. Moreover, final models used in chapters 4 and 5 differed in a number of parameters, while retaining a core of a few variables. This shows how continually reassessing and refining factor inclusion will benefit analysis and data collection practices. Tying modern specimens to additional environmental and climatic factors may be of particular interest for kangaroo conservation efforts in the face of climate change, particularly given the dietary variability of some species. We may also wish to consider how much we want to model differences within groups into differences between species, as this could obscure some variation. For instance, including *ecoregion* minimised the effects of sampling across geographic regions. However, species distributions in part reflect adaptation, so a species living in arid regions has a more coarse diet in part because it lives in arid regions. So by incorporating *ecoregion* effects we may be decreasing our ability to identify causal factors

which effect DMTA data. Balancing how to manage factor inclusion with dietary differentiation may then help refine microwear interpretation.

Modern species provide the essential baselines for palaeodietary inference. Published modern macropodoid data can be found in Prideaux *et al.* (2009), *n*=27, DeSantis *et al.* (2017), *n*=90, as well as chapter 5 here *n*=2598. A challenge for DMTA researchers worldwide is to unite such datasets, particularly where they sample different species or collections. This would provide greater ability to infer diets for extinct species, and understand how DMTA variables correlate to diet. The template developed to minimise inter-microscope differences (chapter 3) should assist in this. Other issues, though, may be harder to overcome. Intraspecific factors can effect DMTA data, but can also be modelled into interspecific comparisons for improved analytical outcomes (chapter 4). Doing so however requires that these characters be scored for each scan, and while some factors (e.g., *tooth, specimen*) can be gleaned from materials and published supplementary data, for the most part such information, particularly regarding *facet*, which is used in numerous final models (chapters 4–5), is lacking or not considered (e.g., Prideaux *et al.* 2009, DeSantis *et al.* 2017). To combine these datasets, then, some factors will need to be further tested to determine the effect of missing data.

With dietary data wanting for many living species of kangaroos, it may be that DMTA data can partly fill this gap. Indeed, many of the difficulties in studying threatened species, such as living in remote areas, or species being rare or reclusive in the wild do not apply to DMTA, where existing museum collections are sufficient, particularly for species which have recently become threatened. Microwear data could fill this void, such as DMTA data for *Dorcopsis vanheurni* (chapter 5), for which our current understanding of diet is limited to presumptions based on morphology and habitat. However, in utilising such data we must be careful to avoid circular reasoning, as modern species such as *D. vanheurni* were also used to define how to interpret macropodid DMTA data.

Modern specimens also offer the opportunity for even greater fine tuning of the dietary signal through more direct dietary inference. Studies of gut or faecal contents are considered the most accurate dietary inference available (chapter 2). In a small number of institutions there exist collections where gut and faecal contents have been collected with associated skeletal material (P. Ungar pers. comm. 17/11/15, T. Dawson pers. comm. 25/8/17). Such specimens would allow direct inference of how diet and microwear correlate, and further may indicate which DMTA variables or combinations of variables best indicate diet. This is particularly applicable given the 'last supper phenomenon' where dental microwear is considered to reflect only the past few weeks of an animal's life (Grine 1986).

Modern macropodoid microwear will obviously be of use in any future DMTA studies of kangaroo diets, but there are still questions of its applicability to related groups. This thesis has operated on the assumption of comparability between macropodine and sthenurine microwear, despite the fact that these subfamilies by definition differ in morphology. Differences in morphology between genera may play a role in shaping microwear patterns (chapter 5). Turning to more distantly related macropodids, such as balbarids or early macropodids, will help further test these assumptions. Even more removed are the many other extinct diprotodontians, for which closely related extant analogues simply do not exist. One possible avenue to consider would be to model differences in morphology into dietary analyses, just as intraspecific differences were modelled into differences between species. Another prospect would be to consider microwear on incisor teeth, which are more similar morphologically between groups. Incisor microwear has already been considered extensively on primates (e.g., Kelley 1990, Ungar 1990, Ungar and Spencer 1999), and rodents (e.g., Teaford and Walker 1983, Hopley *et al.* 2006, Burgman *et al.* 2016). In addition, considering unrelated but morphologically similar forms may provide additional or alternative baselines across broader groups, for example comparing nodern Tapirs with Dirptodontians both of

which have similar bi-lophodont dentitions. Consideration of microwear patterns in groups with convergent molar morphology also may help indicate the broader relationship between morphology and microwear. Within taxonomic groups, considering how diets change over deep time, and how these dietary shifts correspond to changes in morphology may indicate how dietary niches are established and whether these are responsive to broader climatic trends or other factors.

6.4. Conclusions

Diet is one of the most fundamental elements of an animal's biology. Dietary adaptations provide each species with a suite of characteristics in behaviour, morphology and physiology which define how it acquires food throughout its life. However, these are simply guidelines. A chimpanzee has molars that can crack nuts, but it will happily subsist on bananas. Giant pandas have teeth that look much like those of other omnivorous bears, but are bamboo specialists. Understanding diets of species requires direct evidence of what animals actually eat or ate in the past. DMTA is at the forefront of palaeodietary inference. It is non-destructive, cost-effective and relatively simple to undertake, even for large sample sizes. In addition, the methodology is continually advancing through improvements in identifying and then eliminating or incorporating sources of error, as well as fine-tuning how microwear signals relate to diet.

Kangaroos are often seen as the Australian and/or marsupial equivalents of artiodactyl herbivores. However, data presented here suggest that they typically express greater dietary plasticity than placental herbivores. In general, more macropodines are grazers than sthenurines, and more sthenurines are browsers than macropodines, but across the Macropodidae it appears that mixed feeding is the norm rather than the exception, in contrast to bovids (Gagnon and Chew 2000). This plasticity may be one key to their success as a group through the late Cenozoic, allowing some species to vary their diets when beset by

increasingly arid and variable climatic conditions. Dietary flexibility may hold at least some macropodid species in good stead for surviving in an environment increasingly altered by human activities. The capacity also for dietary plasticity in some sthenurines renders the likelihood of climate-mediated extinction across the group unlikely, particularly when the more significant vegetation changes occurred in the wake of, not in the lead up to their extinction around 50,000 years ago (Johnson 2009). This adds to an expanding portfolio of evidence that the impacts of humans, likely via hunting, was instrumental in sthenurine extinctions (e.g., Prideaux 2004, Saltre et al. 2016, Johnson et al. 2016).

This thesis has endeavoured to set a solid platform for investigating and furthering our understanding of the dietary ecology of modern and Pleistocene kangaroos and other marsupials. The methodological challenges that it has met have broader applicability in DMTA elsewhere, as well as in surface metrology more generally. Continuing work will fine tune our understanding of diets and how they are shaped, and are limited only by available collections and the will to undertake such work.

Or so I reckon.

6.5. Acknowledgements

Thanks to Terence Dawson for sharing his wealth of knowledge on kangaroo ecology.

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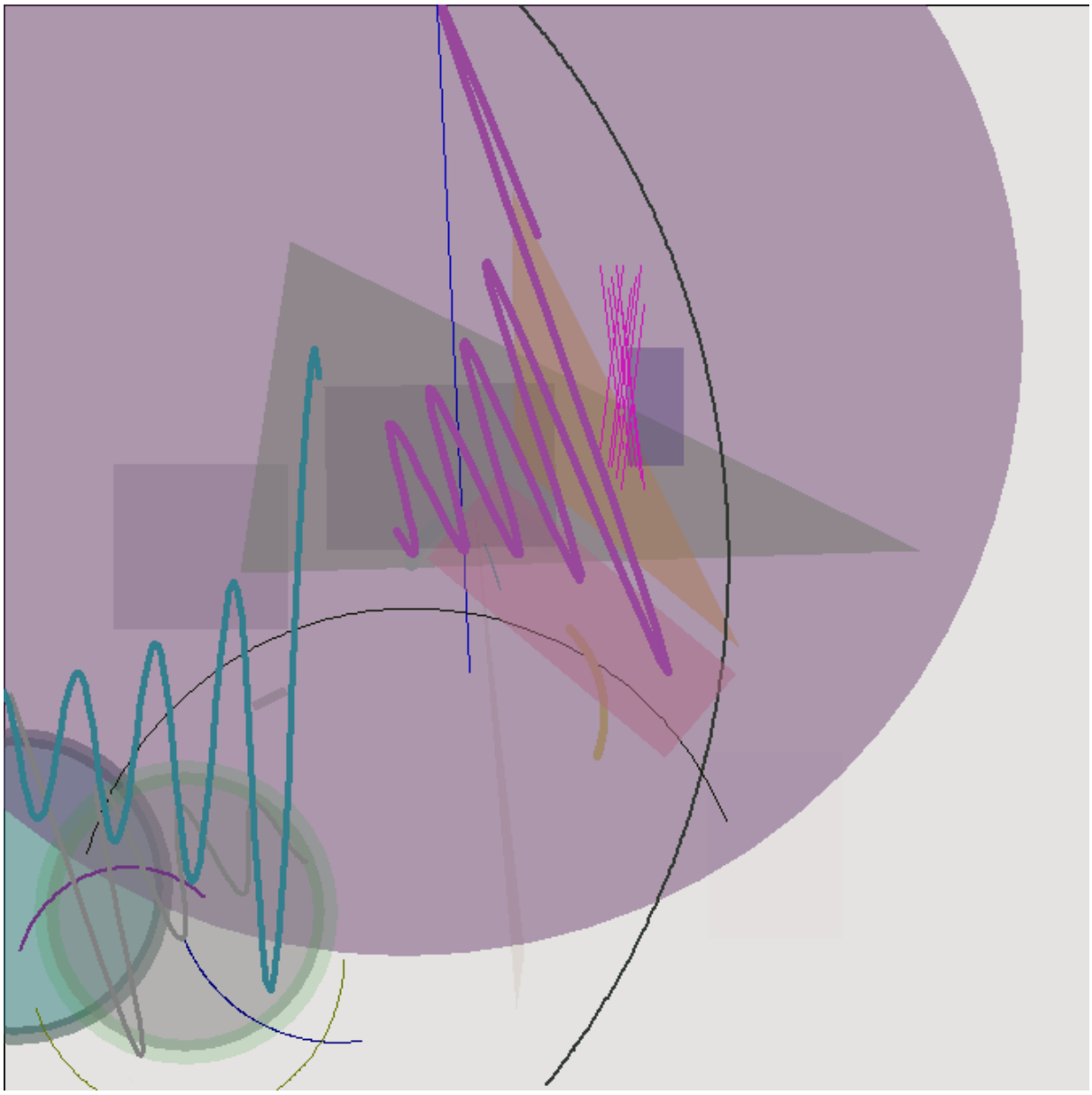
Appendix 1

Kandinsky plot of the final DMTA dataset used in chapter 5. See link for details:

http://giorasimchoni.com/2017/07/30/2017-07-30-data-paintings-the-kandinsky-package/



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