DENTAL MICROWEAR IN RELATION TO DIET IN BLUE DUIKER AND COMMON DUIKER *(Cephalophinae, Bovidae, Mammalia)* IN SOUTH AFRICA

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ABSTRACT

The blue duiker (*Philantomba monticola* Thunberg, 1789) and grey or common duiker (*Sylvicapra grimmia* Linnaeus, 1758) represent two of the three duiker species occurring in southern Africa. Whilst some work has been published on the relationship between dental microwear and diet in a number of antelope species, nothing is documented for duikers.

Scanning electron microscopy (SEM) analysis was used to study dental microwear on the second lower molars of the blue duiker and common duiker specimens collected from the Eastern Cape Province of South Africa and housed in the Shortridge Mammal Collection at the Amathole Museum.

Although there were no significant differences in the dental microwear of the two duiker species, results confirmed that the two are browsing species with a high incidence of pits on their dental surfaces, an attribute due to the presence of fruit in their diet.

Keywords: Dental microwear, scanning electron microscopy (SEM), blue duiker, common duiker, *Philantomba monticola, Sylvicapra grimmia, Bovidae, Cephalophinae.*

INTRODUCTION

Variations in dental microwear have yielded insight into a number of oral processes such as, occlusal relationships and biomechanics of the jaw (Gordon, 1984a, 1984b; Wilkins & Cunningham, 1993), and dietary habits (Smith 1984; Teaford, 1986; Taylor & Hannam, 1987). Probably the most important aspect of microwear analysis is the possibility of using it to deduce the diet of extinct and fossil forms (Grine, 1981; Daegling & Grine, 1987; Waddle, 1988; Van Valkenburgh *et al.*, 1990; Lubell *et al.*, 1994).

Dental microwear analysis has focussed on a number of herbivorous taxa such as Primates and Hyracoidea (Walker *et al.*, 1978; Teaford & Robinson, 1989; Teaford & Runestad, 1992), while there have been a few studies on large antelopes such as waterbuck and kudu (Solounias & Hayek, 1993). No such study on duikers or any other species of small antelopes is documented.

Dental microwear analysis is facilitated by the use of casts and scanning electron microscopy (Murphy, 1982; Roomans, 1984). Analyses range from qualitative to quantitative, and from experimental studies using live animals to comparative studies of museum collections (Teaford, 1988). Teaford & Oyen (1989) state that the process of taking dental impressions from live animals is a difficult one and that it presents problems different from those encountered when working with museum material. However, Teaford & Runestad (1992) stress the importance of using museum specimens collected from the same area at the same time unless the effects of spatial and temporal variation are being investigated.

Several microwear features have been correlated with dietary variations (Covert & Kay, 1981; Teaford & Runestad, 1992; Lukacs & Pal, 1993). Gordon (1982) places these features into three categories, striations or scratches, pits, and gouges. The distinction between pits and scratches can be made through the use of a cut-off point in the range of length to width ratio (Teaford, 1985; Daegling & Grine, 1994) or, by subjective determination (Grine, 1986). Generally, scratches are linear depressions whose length is always greater than breadth (Gordon, 1982). Lengths and breadths of pits are approximately equal, and gouges are usually broader, strongly curved, and often S-shaped (Gordon, 1982).

Microwear features do not necessarily reflect specific food items, but rather the mechanical properties of the items or the constituents of the items (Grine, 1986; Teaford & Robinson, 1989). Therefore, foods with similar mechanical properties might be expected to produce similar microwear patterns (Daegling & Grine, 1994). Microwear patterns have been used to differentiate browsers from grazers (Walker *et al.*, 1978; Teaford, 1985; Mainland, 1998), and frugivores from folivores (Teaford & Walker, 1984; Teaford & Runestad, 1992).

The diet of the blue duiker varies throughout its range (Faurrie & Perrin, 1993) and comprises mainly fallen leaves and fruits (Bowland, 1990; Hanekon & Wilson, 1991). Whereas, Dubost (1984) found a high occurrence of fruit in its diet and described them as frugivorous, Bowland (1990) considered blue duiker to be folivorous on account of their diet comprising mainly leaves. In a recent dietary classification of African bovidae, Gagnon & Chew (2000) state that with the exception of common duiker, all duiker species are frugivorous.

The diet of common duiker consists mainly of forage of various dicots, twigs, flowers and some fruit (Wilson & Clarke, 1962; Boomker, 1983; Allen-Rowlandson, 1986, Skinner & Smithers, 1990).

The aim of this study was to determine if dental microwear is a sufficiently refined tool to detect the small differences in the diets of the blue duiker and common duiker. In addition, patterns of dental microwear could become a valuable taxonomic tool in identification of skulls, jawbones and loose teeth of the two species. Accurate identification of species is of fundamental importance in ecological monitoring, assessment, impact and conservation work as it often underpins the data from which subsequent analyses and interpretations are made.

MATERIALS AND METHODS

SAMPLING OF SPECIMENS

Eleven skulls from each of the two duiker species,

Figure 1. The skulls of blue duiker (left) and common duiker (right).

collected from two neighbouring districts in the Eastern Cape Province, and with the closest possible dates of collection, were used for the study. The specimens were obtained from the Amathole Museum

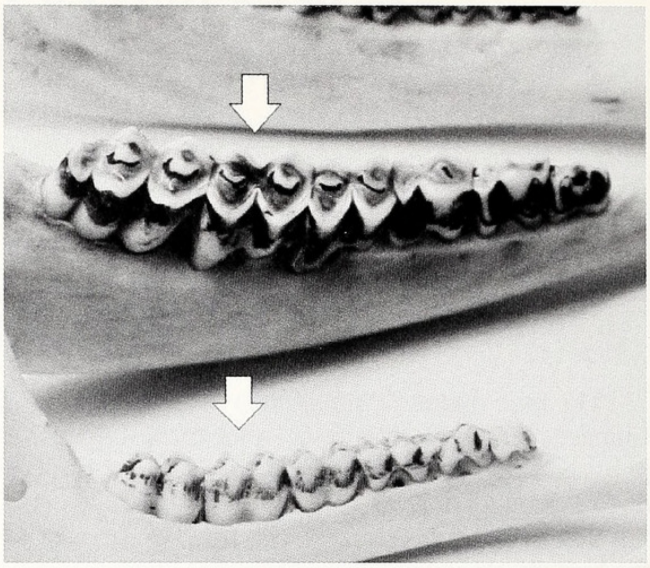


Figure 2. The lower jaws of blue duiker (foreground) and common duiker (background) showing the positioning of the second lower molar.

mammals collection (Appendix 1). This sample size is comparable to the ten used by Teaford (1985), and the eight of Gordon (1984 d).

The second lower molars (Skinner & Smithers, 1990) were selected for SEM analysis because they are placed between two other molars (Fig.2) and therefore the occlusal function is the same on both sides (Rensberger, 1973). They are also smaller in width than the upper molars and they occlude over the entire surface whereas the upper ones have only part of the crown occluded by all the lower ones as a result of the overlap (Butler, 1978).

PREPARATION OF DENTAL REPLICAS

With the use of cotton wool the teeth surfaces were cleaned with water, then ethanol and finally acetone (Rose, 1983). This frees the surface of any dirt, glue, loose matrix or grease (Rose, 1983). Vigorous scrubbing which may create artefactual scratches was avoided (Teaford & Oyen, 1989). After allowing the surface to air dry (Rose, 1983), a thin coating of acetic acid was applied to it, and a wall of Bostik Prestik sticky stuff (Genkem Ltd, England) was built around the tooth. Latex was then poured on top of the acid layer. The acetic acid facilitates rapid and proper setting of the impression material and together with the barrier prevent seepage of the latex (Ryan, 1979).

Preliminary SEM analysis indicated that latex did not pick up the dental impressions, and subsequently its use was abandoned. Molds of the teeth were then made with a mixture of an equal amount of Aquasil smart wetting impression material (S.W.I.M) "base" and Aquasil S.W.I.M " hardener catalyst" (Dentsply / Caulk, Milford DE, USA). As recommended by Rose

(1983) the impression material was applied to the teeth surfaces with disposable plastic syringes. Once the moulds were peeled off the teeth they were allowed to sit in a dust free environment at room temperature for about eight hours, in order to permit total degassing (Grine 1986). This is necessary in order to prevent any artefactual pitting (Gordon 1984 c). Initially epoxy-resin casts were made from the moulds as was the case in several studies (Gordon, 1984 d; Bullington, 1988; Teaford & Runestad, 1992). However, this was discontinued, as a result of failure of the casts to separate cleanly from the moulds, after setting. Rose (1983) states that clean separation is an important requirement for suitability of any casting material, which if not met may result into formation of artefacts. This led to a comparative examination of SEM images of teeth and images of their Aquasil S.W.I.M moulds. Based on the similarity of these images it was decided to use the original Aquasil moulds which once made and allowed time to degass were sputter-coated with gold prior to SEM analysis (Echlin, 1978).

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

Approximately two hours after coating the moulds they were carefully orientated in a particular angle, (long axes of moulds uniformly placed) and mounted on marked stubs. Care was taken to avoid any direct contact with the mould surfaces (Rose, 1983).

They were examined in a Jeol JSM 840 scanning electron microscope. Teeth were rotated in various positions to have an overview of features, and comparative micrographs were taken (Crompton & Kielan-Jaworowska, 1978). Sets of micrographs at magnifications of 130x and 450x were taken, but only the latter were used for analyses.

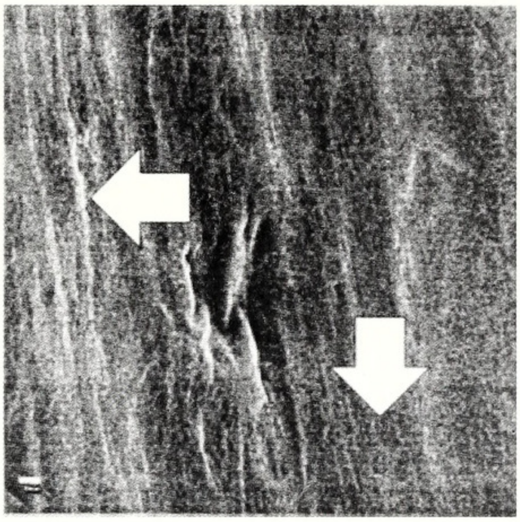
DATA COLLECTION AND ANALYSES

Data were collected from eleven micrographs for each of the two duiker species. These were a total of twenty-two micrographs taken from similar occlusal facets of the second lower molars. With the use of a B41420/3 illuminated magnifier all identifiable pits and scratches in an area of 16 cm^2 at the centre of each micrograph (of 450 X magnification) were counted and recorded. Gouges were subsumed as scratches (Grine, 1986). Pits and scratches were identified independently by subjective determination following the method of Gordon (1982), rather than by imposing an arbitrarily set length to width ratio on the features. In order to ensure that features are correctly categorised, only those which could clearly be identified were recorded. It has been stated (Gordon, 1982; Teaford and Walker, 1984) that, because of the overlap and large numbers of features per field it is not always possible to record every feature.

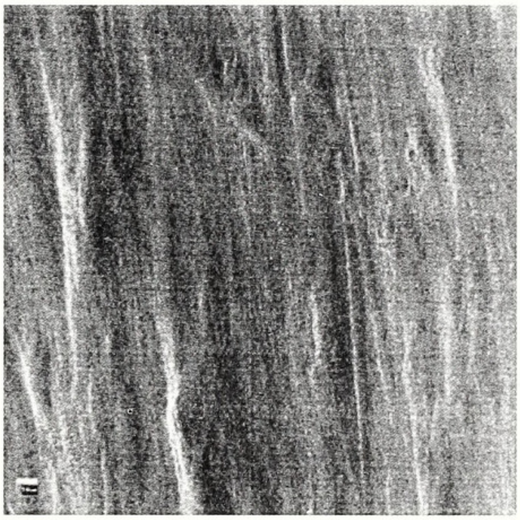
The number of microwear features per field of examination was compared between samples using the Mann-Whitney statistic or t- test. Chi-square analysis was used to test for interspecific differences in the proportions of pits and scratches (Zar, 1996).

RESULTS

Three types of features; scratches, pits and gouges were identified as occurring on all teeth of the blue duiker and common duiker. Representative images of casts from the teeth of the blue duiker and common duiker are shown in the micrographs (Figs. 3A and B).



A



B

Figure 3. Scanning electron micrographs taken from the occlusal surfaces of the second lower molars of common duiker (A) and blue duiker (B). The horizontal arrow indicates a scratch and the vertical arrow a pit. Magnification is 450X.

Table 1. Numbers, ratios, percentages and results of statistical comparisons of microwear features on teeth of blue and common duiker.

Dental microwear features	Blue duiker (n =11)	Common duiker (n =11)	Statistical results
Mean number of scratches per 16 cm^2	79 (15%)	84 (14.9%)	
Mean number of pits per 16 cm^2	449 (85%)	481 (85.1%)	
Ratio of Pits : Scratches	5.7 : 1	5.5 : 1	Insignificant Interspecific diff. (Chi-Square; p = 0.96)

The total number and percentages of pits and scratches recorded on the teeth of the blue duiker and common duiker were similar and for both species there were more pits than scratches on the dental surfaces (Table 1).

The chi-square analysis showed no significant interspecific differences in the proportions of pits and scratches between duiker teeth ($p = 0.96$).

Interspecific comparison of the mean number of features per field indicated no significant differences ($p = 0.53$, Mann-Whitney). Intriguingly however, significant intraspecific differences were shown to exist between the number of pits and scratches per field in both the blue duiker ($P < 0.0001$, t-test) and common duiker ($P < 0.0001$, t-test).

The lowest recorded number of features in a 16 cm square field (from a micrograph of 450 X) on common duiker teeth was 21 compared with 9 in a similar field on blue duiker teeth. The highest number recorded, in the same size of fields for common duiker and blue duiker were 94 and 97 respectively.

DISCUSSION

It is established that the diets of grazers and browsers result in different patterns of molar microwear (Walker *et al.*, 1978; Teaford, 1985; Solounias & Hayek, 1993; Mainland, 1998). Browsers are characterised by many pits and few scratches, while grazers have many scratches and few pits (Solounias & Hayek, 1993). A frugivorous diet results in teeth with a high density of pits (Teaford & Walker, 1984; Teaford & Runestad, 1992) and hard fruit eaters have wider pits than soft fruit eaters (Teaford, 1985; Teaford & Runestad, 1992). Blue duiker and common duiker have been described as browsers by Boomker (1981), Wilson (1966), Bowland (1990) and Bowland & Perrin (1998) and this is supported by the type of microwear exhibited on their dental surfaces. The molars of both species are predominantly pitted which is characteristic of browsers.

Covert & Kay, (1981) and Peters (1982) attribute the differential wear on teeth of grazers (many scratches and few pits) to the opaline phytoliths in grasses. Baker *et al.,* (1959) and Kay & Covert, (1983) demonstrated that wear caused by the opaline phytoliths and that of gritty diets are essentially similar. Blue duiker eat fallen leaves, fruit, and flower (Skinner & Smithers, 1990; Faurie & Perrin, 1993; Bowland & Perrin, 1998) which are ingested along with accompanying debris. As a result, it might be expected that blue duiker dental wear would be closer to that of grazers, but the results from the present study suggest that this is not the case. It is possible that utilisation of fruit by both duiker species explains this. Teaford & Walker, (1984) and Teaford & Runestad (1992) relate a high incidence of pits to a frugivorous diet. It is therefore likely that the dental effects of eating fruits in both duiker species overshadows any minor feeding habit differences.

The significant intraspecific differences (between similar sites on the molars of the same species) in the number of features per field is interesting. Although every effort was made to ensure that specimens for the study were collected from the same area at the same time, this was not entirely possible. All specimens had been collected from Peddie and King William's Town districts, which are neighbouring jurisdictional areas of the Eastern Cape province. At the time of collecting the specimens, both these districts were described as having similar vegetation types, which was a combination of valley bushveld and Eastern province thornveld (Comins, 1962; Acocks, 1975). All specimens were collected between 25 May 1948 and 5 April 1949. However, most specimens were collected during autumn and winter (Appendix 1). Despite the use of only adult specimens (of unknown ages) in the study, age was not fully controlled for, and therefore may also have been a variable determining the results. Although the factor of gender is not mentioned in the literature, in this study only three specimens were of unknown sex, and males and females were spread evenly throughout all categories (species and localities) (Appendix 1).

Gordon (1982) postulates that the recognised types of microscopic abrasion features are not intrinsically different, but rather manifestations of different degrees of shear and compression subjected to the agents which produce microwear. According to this view, pits and scratches are found at opposite ends of a continuum of surface wear phenomena, such that the decision about where to make the division is always arbitrary (Gordon 1988). Different cut-off points have been used. Daegling & Grine (1994) defined pits as those features with a length-width ratio of 4:1 or below, while Teaford & Walker (1984) and Teaford (1985) assigned to pits a ratio of 10:1 and anything above to scratches. Subsequent assessment of the features indicated that, those features recognised as pits in this study possessed length to width ratios of about 4:1 and below. In an analysis of feature dimension ratios, Solounias & Hayek (1993) concluded that the best diagnostic method of tooth microwear analysis utilizes the number of pits smaller than or equal to the ratio four (length over width), the number of scratches between four and one hundred in length to width ratio, and that of gouges greater than one hundred in length to width ratio.

The data for this study were from microwear counts. Microwear feature densities and relative abundance have widely been used to detect dietary differences among closely related species (Teaford & Walker, 1984; Teaford & Runestad, 1992; Solounias & Hayek, 1993) however, feature dimensions are also equally important (Gordon, 1982). Robson & Young, (1990) state that microwear feature dimensions rather than feature densities and relative abundances, may be the most suited for investigating diet differences of closely related species. On the other hand, Teaford & Runestad (1992) describe scratch widths as poor indicators of dietary differences.

In conclusion, dental microwear cannot be used to separate the blue duiker and common duiker. However, the abundance of pits on the occlusal surfaces supports the observation that both are browsers, with fruit as part of their diets.

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APPENDIX 1

Information accompanying the blue and common duiker museum specimens whose molars were used in the dental microwear analyses.

Specimen	Catalogue Number	Sex	District of origin	Date collected
Blue duiker	KM 15056	M	King William's Town	25 April 1948
Blue duiker	KM 15060	?	King William's Town	25 May 1948
Blue duiker	KM 15082	F	King William's Town	30 July 1948
Blue duiker	KM 15037	M	King William's Town	27 June 1949
Blue duiker	KM 15055	M	King William's Town	06 May 1948
Blue duiker	KM 15558	?	King William's Town	08 May 1949
Blue duiker	KM 15559	?	King William's Town	27 June 1949
Blue duiker	KM 15052	M	Peddie	04 July 1948
Blue duiker	KM 15053	M	Peddie	01 July 1948
Blue duiker	KM 15062	M	Peddie	04 July 1948
Blue duiker	KM 15081	M	Peddie	19 July 1948
Common duiker	KM 15098	F	King William's Town	02 March 1949
Common duiker	KM 15099	F	King William's Town	05 April 1949
Common duiker	KM 15100	F	King William's Town	07 April 1949
Common duiker	KM 15101	F	King William's Town	17 January 1949
Common duiker	KM 15102	M	King William's Town	17 January 1949
Common duiker	KM 15103	M	King William's Town	09 July 1948
Common duiker	KM 15129	M	King William's Town	16 May 1948
Common duiker	KM 15138	M	Peddie	28 August 1948
Common duiker	KM 15162	M	Peddie	02 August 1948
Common duiker	KM 15163	M	Peddie	08 August 1948
Common duiker	KM 15164	F	Peddie	08 August 1948

KEY

KM Amathole Museum (Formerly Kaffrarian museum)

? Not given

F Female

M Male

King William's Town (3327 CD) and Peddie (3327 AA) districts are in the Eastern Cape Province of South Africa.