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Geographic distribution of C$_3$ and C$_4$ grasses recorded from stable carbon isotope values of bone collagen of South Australian herbivores

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Abstract. Cortical bone samples were collected from marsupial and eutherian herbivores at five field sites along a 1275-km south–north transect from temperate coastal to arid interior South Australia in order to address variability in stable carbon isotope composition. Collection sites were located along the eastern border of the state. Mean annual rainfall along the transect ranges from 700–800 mm at coastal Mount Gambier to 150–175 mm at Cordillo Downs in the north-east corner of the state. Bone collagen carbon isotope values become more positive towards the arid north in relation to increasing quantities of C$_4$ grasses. Thus, stable carbon isotope analysis of bone specimens provides a method to address dietary selection and dietary variability in Australian herbivores. In addition, isotopic analyses of archaeological and palaeontological bones and teeth can be used to address changes in Quaternary climate and vegetation distributions in Australia.

Introduction

The stable carbon isotopic composition of an animal’s tissues reflects the isotopic composition of ingested foods. In bone, dietary information is recorded by carbon isotope ratios ($\delta^{13}C$) in collagen and the carbonate component of the inorganic calcium phosphate portion of bone and tooth mineral (Schwarcz 1991; Pate 1994). Due to slow biochemical turnover rates, bone stable isotope values are related to long-term patterns of food consumption and habitat use. In most vertebrates, stable carbon isotopes provide information about lifetime dietary averages (Stenhouse and Baxter 1979). As the carbon isotopic composition of soils and plants show spatial variations within regional food webs, herbivore bone has the potential to provide an average isotopic signal for particular environments (Ehleringer and Cooper 1988; Korner et al. 1988; Cormie and Schwarcz 1994).

Terrestrial plants employ three distinct photosynthetic pathways (C$_3$, C$_4$, CAM) that produce characteristic $\delta^{13}C$ tissue values (O’Leary 1988). C$_3$ plants include all trees, most shrubs and herbs, and temperate cool season grasses. C$_4$ plants include tropical and warm season grasses and a few shrubs in the families Euphorbiaceae and Chenopodiaceae. CAM photosynthesis is restricted to arid-land succulents, e.g. cacti, agave and some euphorbs (Salisbury and Ross 1992).

Plants using the C$_4$ (Hatch–Slack) pathway have markedly higher $\delta^{13}C$ values than plants employing the C$_3$ (Calvin–Benson) pathway. Mean $\delta^{13}C$ values of $-13.1 \pm 1.2\%$ for C$_4$ plants and $-27.1 \pm 2.0\%$ for C$_3$ plants are reported for a large number of species grown under a variety of conditions (O’Leary 1988). Carbon isotope tissue ratios vary from $-7$ to $-16\%$ in C$_4$ plants and from $-20$ to $-35\%$ in C$_3$ plants (Deines 1980; Ehleringer 1989). CAM plants exhibit two distinct photosynthetic phenotypes (Teeri 1982). CAM plant $\delta^{13}C$ tissue values range from $-10$ to $-14\%$ when essentially all uptake of atmospheric CO$_2$ during growth occurs at night via crassulacean acid metabolism or from $-24$ to $-30\%$ when most CO$_2$ uptake occurs in daylight via C$_3$ photosynthesis.

These distinct isotopic signatures in C$_3$ and C$_4$ plants are passed on to the tissues of consumers with a small change or fractionation related to biochemical processes within the animal. Field studies in South Africa with large herbivores report fractionations of $+5\%$ for animals consuming C$_3$ plants and $+6\%$ for animals consuming C$_4$ plants (van der Merwe et al. 1988; van der Merwe 1989). Thus, employing mean stable carbon isotope values for C$_3$ and C$_4$ plant tissues, grazers with pure C$_4$ plant diets would be expected to have mean bone collagen $\delta^{13}C$ values of about $-7.1\%$, while grazers with pure C$_3$ plant diets would have mean values of about $-22.1\%$. Animals with mixed C$_3$ and C$_4$ diets will have intermediate bone collagen $\delta^{13}C$ values.

Hattersley (1983) reports the geographic distribution of C$_3$ and C$_4$ grasses in Australia in relation to climatic zones. The southern temperate regions of South Australia are dominated by C$_3$ grasses, whereas C$_4$ grasses become dominant in the arid northern areas. This paper addresses the relationship between the distribution of C$_3$ and C$_4$ grasses and carbon isotope values of bone collagen stable of herbivore consumers at five collection sites in South Australia.
Materials and methods

Cortical bone samples were collected from modern marsupial and eutherian herbivores at five field sites along a 1275-km south–north transect skirting the eastern border of South Australia. Collection sites included Mount Gambier, Karte, Plumbago, Innamincka, and Cordillo Downs (Fig. 1). Samples were collected within a 30-km radius of each site. The transect extends from 38°00'S to 26°45'S and mean annual rainfall ranges from 700–800 mm at Mount Gambier to 150–175 mm at Cordillo Downs. Percentage of C₄ grasses in the collection areas ranges from 23% at Mount Gambier to 78% in the north-eastern Lake Eyre region (Table 1).

Herbivores sampled included western grey kangaroos (Macropus fuliginosus), red kangaroos (M. rufus), sheep (Ovis aries) and cattle (Bos taurus). With regard to diet, all of these animals are predominantly grazers (Barker 1987; Hofmann 1989). In arid habitats and during droughts, however, kangaroos, sheep, and cattle may include greater quantities of browse in their diets (Ellis et al. 1977; Squires 1981).

Collagen was extracted from whole bone according to the methods employed by Sealy (1986). Whole bone chunks weighing 1–2 g were scraped clean with a scalpel, washed in an ultrasonic cleaner, and defatted in a chloroform : methanol : water (2:1:0.8, v:v:v) solution. Defatted bone was demineralised in dilute HCl. Extracts were soaked and washed thoroughly in distilled water following treatments. The remain-

![Fig. 1. Map showing location of the five South Australian sample collection sites: (1) Mount Gambier, (2) Karte, (3) Plumbago, (4) Innamincka, (5) Cordillo Downs and associated geographic subdivisions indicating abundance of C₃ and C₄ grasses in relation to climate (after Hattersley 1983). Geographic subdivisions: SE = South-eastern, MY = Murray, EN = Eastern, LE = Lake Eyre.](image-url)
ing organic component was oven dried at 35°C, and stable carbon isotope values were determined by mass spectrometry. Analytical precision was better than ± 0.1‰.

**Results**

Stable carbon isotope values of bone collagen for both marsupial and eutherian herbivores become more positive towards the arid north in relation to increasing quantities of C4 grasses. (Table 2). At Mount Gambier, herbivore bone collagen δ13C values suggest diets based predominantly on C3 plants, and mean δ13C values for sheep/cattle and kangaroos are 1.1 and 2.7‰ more negative, respectively, than the –22.1‰ bone collagen value predicted for large herbivores with pure C3 diets. Pate and Schoeninger (1993) noted similar bone collagen δ13C values in Tammar wallabies (*Macropus eugenii*) and koalas (*Phascolarctos cinereus*) collected off the coast of south-eastern South Australia at Kangaroo Island.

At the two arid collection sites where both marsupial and eutherian grazers were sampled, kangaroos have more negative bone collagen δ13C values than cattle and sheep, suggesting the inclusion of greater quantities of C4 grasses in the diets of the marsupial herbivores. On the basis of bone collagen δ13C values, kangaroo diets consist of about 41 and 53% C4 grasses at Plumbago and Innamincka, respectively; whereas, cattle and sheep from the same sites have a 27 and 40% C4 grass component. The greatest quantity of C4 grasses observed in herbivore diets occurs at Cordillo Downs where cattle have mean bone collagen δ13C values of –13.0‰, equating to an average diet of about 63% C4 grasses. Bone collagen δ13C values for Plumbago, Innamincka, and Cordillo Downs also indicate that herbivore diets are more variable in the arid interior regions of the state in comparison to the two most southern collection sites.

**Discussion**

The variability in modern bone collagen δ13C values observed along the transect, suggests that stable carbon isotope analyses can contribute to studies of dietary selection and dietary variability in Australian herbivores. A majority of the variability in herbivore bone collagen δ13C values can be explained by the geographic distribution of C3 and C4 grasses.

**Table 1.** Percentage of C4 grasses at five South Australian collection sites along a south–north transect from temperate coast to arid interior

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Location</th>
<th>Mean annual rainfall (mm)</th>
<th>Geographic subdivision</th>
<th>Percentage C4 grasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mount Gambier</td>
<td>37°56’S, 140°47’E</td>
<td>700–800</td>
<td>Southeast</td>
<td>23</td>
</tr>
<tr>
<td>Karte</td>
<td>35°04’S, 140°42’E</td>
<td>300–400</td>
<td>Murray</td>
<td>47</td>
</tr>
<tr>
<td>Plumbago</td>
<td>32°04’S, 139°53’E</td>
<td>200–250</td>
<td>Eastern</td>
<td>69</td>
</tr>
<tr>
<td>Innamincka</td>
<td>27°56’S, 140°47’E</td>
<td>150–175</td>
<td>Lake Eyre</td>
<td>78</td>
</tr>
<tr>
<td>Cordillo Downs</td>
<td>26°45’S, 140°40’E</td>
<td>150–175</td>
<td>Lake Eyre</td>
<td>78</td>
</tr>
</tbody>
</table>

**Table 2.** Mean bone collagen stable carbon isotope results (δ13C) for South Australian marsupial (kangaroos) and eutherian (cattle and sheep) herbivores collected along a south–north transect from temperate coast to arid interior

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Marsupial herbivores</th>
<th>Eutherian herbivores</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mount Gambier</td>
<td>–24.8 ± 0.8 (6)</td>
<td>–23.2 ± 1.2 (17)</td>
<td>–23.6 ± 1.2 (23)</td>
</tr>
<tr>
<td>Karte</td>
<td>–21.6 ± 0.7 (7)</td>
<td>–21.8 ± 0.8 (7)</td>
<td>–21.7 ± 0.7 (14)</td>
</tr>
<tr>
<td>Plumbago</td>
<td>–17.6 ± 2.4 (13)</td>
<td>–18.9 ± 2.2 (8)</td>
<td>–18.2 ± 2.4 (21)</td>
</tr>
<tr>
<td>Innamincka</td>
<td>–15.4 ± 1.2 (12)</td>
<td>–16.8 ± 1.5 (8)</td>
<td>–16.2 ± 2.3 (20)</td>
</tr>
<tr>
<td>Cordillo Downs</td>
<td>no data</td>
<td>–13.0 ± 2.9 (8)</td>
<td>–13.0 ± 2.9 (8)</td>
</tr>
</tbody>
</table>
be attributed to inclusion of varying amounts of C\textsubscript{3} and C\textsubscript{4} grasses in animal diets.

The more negative bone collagen $\delta^{13}\text{C}$ values observed in the southern Mount Gambier herbivores relative to those inferred from mean global plant $\delta^{13}\text{C}$ tissue values and analyses of South African herbivore bones provide further support for the use of local faunal controls in stable isotope ecological research. The observed differences in stable carbon isotope ratios of bone collagen in South Australia may be attributed to (i) different fractionation values from plants to herbivore tissues and/or (ii) local or regional variations in C\textsubscript{3} plant $\delta^{13}\text{C}$ tissue values that are passed on to higher trophic level consumers.

In addition, aridity causes minor variations in the stable carbon isotope composition of plant tissues. In arid-land habitats soil moisture stress leads to a reduced discrimination against $^{13}\text{C}$ during C\textsubscript{3} plant photosynthesis (Farquhar et al. 1987; Ehleringer and Cooper 1988; Ehleringer 1989). Ehleringer and Cooper (1988) report a 1–2.5\% intraspecific increase in the leaf $\delta^{13}\text{C}$ values of desert perennials along a decreasing soil moisture cline from wash to slope microhabitats. The lower amounts of C\textsubscript{4} grasses observed in the diets of cattle and sheep versus kangaroos in the northern arid zones, may be due to dietary supplements of C\textsubscript{3} hay provided to domestic range stock during periods of food shortage.

The bone collagen stable carbon isotope data for Innamincka and Cordillo Downs suggest that there is greater variability in the abundance and distribution of C\textsubscript{4} grasses within the Lake Eyre subdivision than that reported by Hattersley (1983). Hattersley (1983: 114) provides an average figure of 78\% C\textsubscript{4} grasses for the large Lake Eyre subdivision. The stable isotope data suggest that the percentage of C\textsubscript{4} grasses increase from the southern to the northern regions of the Lake Eyre subdivision.

Stable isotope analyses of plant and animal tissues are providing additional methods to study modern ecological systems (Rundel et al. 1989). Furthermore, isotopic analyses of bones and teeth from archaeological and palaeontological sites are assisting with Quaternary palaeoclimatic and palaeoecological reconstructions and inferences regarding prehistoric human dietary variability and landscape use (MacFadden and Bryant 1994; Pate 1994, 1997, 1998; Noble 1995; Quade and Cerling 1995; Grocke 1997). With regard to stable carbon isotopes, $\delta^{13}\text{C}$ values in bones and teeth can contribute to palaeoecological research addressing temporal changes in C\textsubscript{3} and C\textsubscript{4} plant distributions and associated changes in faunal distributions during glacial and interglacial periods.

Acknowledgments

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