TWENTY-FOUR

Stable Carbon and Oxygen Isotope Analysis of Marmot Cheek Teeth from the Pit Locality

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The Pit locality within Porcupine Cave, Park County, Colorado, spans at least two glacial-interglacial cycles, the upper of which dates to somewhere between 780 and 900 Ka (Bell and Barnosky, 2000). The deposits probably correlate with parts of oxygen isotope stages 21 and 22, or, less likely, 19 and 20 (see figure 7.5). Recent work has demonstrated that carbon and oxygen isotope values found in the tooth enamel of large mammals (*Equus* and *Cuvieronius;* MacFadden, 2000) and small mammals (*Thomomys* and *Geomys;* Rogers and Wang, 2002) correlate well with the marine isotope record. This chapter reports isotopic signatures of marmot tooth enamel from stratigraphically superposed levels in the Pit in hopes of refining correlations and assessing vegetational change as indicated by diet.

The carbon isotope values in the enamel of fossil teeth are useful in determining paleodiet because different photosynthetic pathways impart different $^{13}C/^{12}C$ ratios to different plants, and these ratios are ultimately reflected in the animals that eat the plants (DeNiro and Epstein, 1978; O'Leary, 1988; Ehleringer et al., 1991; Quade et al., 1992; Ehleringer and Monson, 1993; Cerling et al., 1999; Feranec and MacFadden, 2000). Although postdepositional diagenesis can overprint carbon isotope values in bone (Schoeninger and DeNiro, 1982), tooth enamel is not prone to diagenetic alteration, and thus it reliably reflects isotope values derived from feeding (Quade et al., 1992; Wang and Cerling, 1994; Koch et al., 1997). As a general guide, grazers are predominantly C_4 feeders, browsers are predominantly C_3 foragers, and intermediate and crassulacean acid metabolism (CAM) feeders utilize both C_3 and C_4 in modern ecosystems. In addition to their use in determining paleodiet, carbon isotope values fluctuate during glacial-interglacial cycles and so can be used for correlating the terrestrial and marine isotope records (Raymo et al., 1997; MacFadden, 2000).

Oxygen isotope ratios in mammalian tooth enamel have been shown to reflect the isotopic ratio of ingested waters (Land et al., 1980; Longinelli, 1984; Luz et al., 1984; Koch et al., 1989). Luz et al. (1984) and Koch et al. (1989) suggested that the oxygen isotope composition of teeth is dependent on the isotopic composition of the water ingested, the consistent fractionation of oxygen isotopes between the body water and the enamel, and the metabolism of the particular animal. If comparisons are made using the same species over time, the fractionation and metabolism effects should be the same for all samples. Therefore the tooth enamel oxygen isotope values should represent changes in the isotopic composition of the water, which is temperature dependent, and not changes in the metabolism or fractionation of the animals being sampled (Rozanski et al., 1992, 1993). Because oxygen isotope values of meteoric waters should be temperature dependent, this method should allow for discrimination between glacial stages (cold periods) and interglacial stages (warm periods).

Body size of various taxa has been a significant concern when studying the oxygen isotope composition of tooth enamel. Bryant and Froelich (1995) suggested that the largest animals in a fauna are likely to be the best markers of paleoclimate. Similarly, Luz et al. (1984) suggested that the mammals best suited for this type of analysis are those that are obligate drinkers and have a low metabolism. Marmots are not the largest animals in the fauna within the Pit, nor do they continually drink or have a constant low metabolism throughout the year. However, they are among the larger animals that occur abundantly in all levels, and with sufficient frequency for reliable statistical analysis to be performed. Marmot life histories include birth of most animals in early spring and hibernation from early fall through winter, generally about eight months per year (Frase and Hoffmann, 1980). (Additional information on the life history of marmots is given in chapter 25.) Because of this life history, the possibility for erroneous isotopic interpretation owing to seasonal differences among individuals in diet and water intake is minimal. Additionally, Lindars et al. (2001) and Rogers and Wang

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(2002) have demonstrated good paleoclimate signals in tooth enamel from rodents much smaller than marmots.

Materials and Methods

Within the Pit sequence, level 1 is the youngest and level 7 is the oldest of the levels analyzed here. *Marmota* teeth were analyzed from each of these levels (figure 24.1). See Barnosky et al. (1996), Wood and Barnosky (1994), Bell and Barnosky (2000), and Chapters 7 and 23 for details of the stratigraphy. The method for stable isotope sampling and preparation followed that of MacFadden and Cerling (1996) and Koch et al. (1997). Sampling focused on, but was not confined to, the fourth premolar and third molar because these teeth are among the last to mineralize and erupt (Munson, 1984), thereby maximizing the probability that the metabolic pathways that ultimately determine isotopic signatures were those of adult animals. Sampling involved removing all nonenamel portions of the tooth so that only pristine enamel remained. The enamel was then crushed into a fine powder using a mortar and pestle. The pristine enamel powder was first treated with 35% hydrogen peroxide for 24 hours to remove organics. It was then decanted and washed with distilled water, and soaked in 0.1 N acetic acid for 24 hours to remove any diagenetic carbonate. The sample was again decanted and washed with distilled water, rinsed with 100% ethyl alcohol, and dried overnight.

Following the treatment, the samples were analyzed using an Isocarb automated carbonate preparation system attached to a Micromass Optima gas source mass spectrometer (GV Instruments, Manchester, United Kingdom, for both instruments) at the Department of Earth and Ocean Sciences, University of California, Santa Cruz. During analysis the samples were dissolved in 100% phosphoric acid at 90°C to create CO_2. The results were compared using the following equation:

$$X = [(R_{sample}/R_{standard}) - 1] \times 1000,$$

where X is the $\delta^{13}C$ or $\delta^{18}O$ value and $R = {}^{13}C/{}^{12}C$ or ${}^{18}O/{}^{16}O$. All isotope values are reported relative to the V-PDB. The precision for the analysis was ± 0.1‰. A total of 20 samples were collected, prepared, and analyzed. For the analysis of diet, values more negative than –8.0‰ were interpreted to indicate dominantly C_3 feeders, values more positive than –2.0‰ were interpreted to signal dominantly C_4 feeders, and values between –2.0‰ and –8.0‰ were assumed to reflect intermediate feeding strategies.

Mean differences in carbon and oxygen isotope values of *Marmota* tooth enamel between levels and from glacial and interglacial stages in the Pit were compared using single-factor analysis of variance (ANOVA) and Tukey honest significant difference (HSD) tests. (Tukey HSD tests are similar to *t*-tests but take into account multiple comparisons and are generally more conservative.) Because the requirements of ANOVAs (normal distribution, homogeneity of variances) may have been violated by such small sample sizes, a nonparametric Kruskal-Wallis test was also performed. The results from this nonparametric test were the same as those from the ANOVA; therefore only the results from the parametric ANOVA are reported. Statistical analyses were performed in Microsoft Excel 2000 and JMP IN 4.0 (SAS Institute, Inc., Cary, North Carolina), with significance set at $p < 0.05$.

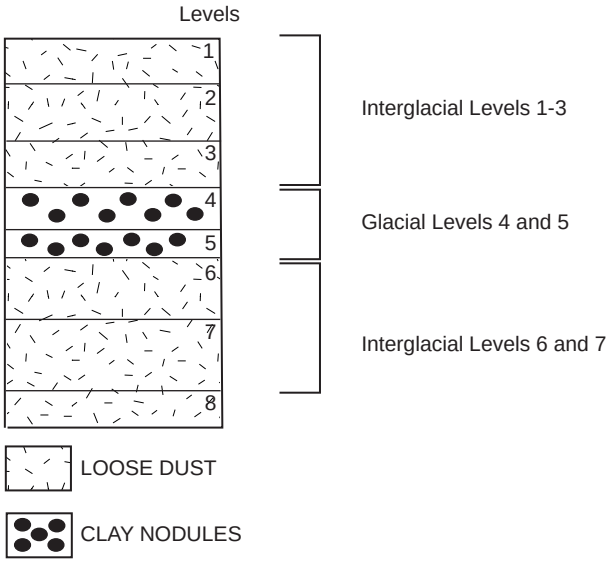

FIGURE 24.1 Schematic stratigraphic column within the Pit sequence showing sediment type. Marmot teeth were obtained from levels 1–7. Glacial and interglacial groupings of levels are indicated. For a more complete description of the stratigraphy within the Pit, see Barnosky and Rasmussen (1988), Wood and Barnosky (1994), Bell and Barnosky (2000), and chapters 2, 7, and 23. (Modified from Wood and Barnosky [1994] and Bell and Barnosky [2000].)

Results

The statistical analysis of isotopic values was conducted on two different scales. The first compared isotopic values between all sampled stratigraphic levels within the Pit, irrespective of whether the level had been assigned to a glacial or an interglacial by evidence independent of isotopes (Wood and Barnosky, 1994; Barnosky et al., 1996; Bell and Barnosky, 2000; Barnosky et al., chapter 23). The other comparison lumped samples into interglacial levels 1–3, glacial levels 4 and 5, and interglacial levels 6 and 7. The expectation was that more differences might be evident between major climatic episodes than within them.

Statistical Analysis by Stratigraphic Level

The carbon and oxygen isotope values for each specimen sampled are presented in table 24.1. Mean stable oxygen isotope values for each level are as follows: level 1, –0.4‰; level 2, 2.2‰; level 3, 1.4‰; level 4, 0.9‰; level 5, 0.4‰; level 6, 4.3‰; level 7, –0.3‰ (figure 24.2). The ANOVA and Tukey

TABLE 24.1
Oxygen and Carbon Isotope Values for *Marmota* Tooth Enamel

Sample Number	*UCMP Specimen Number*	*Level*	*Tooth Sampled*	$\delta^{18}O$	$\delta^{13}C$
RSF 001	181095	1	LM1 or LM2	–0.1‰	–11.8‰
RSF 002	181120	1	Rm1 or Rm2	–0.1‰	–11.7‰
RSF 003	181117	1	Rm1 or Rm2	–0.1‰	–12.5‰
RSF 004	181180	2	Lm1 or Lm2	1.5‰	–11.8‰
RSF 005	181176	2	Rp4	5.0‰	–10.3‰
RSF 006	181194	2	LM3	0.1‰	–11.4‰
RSF 007	181216	3	LP4	1.6‰	–10.6‰
RSF 008	93173	3	Lm3	2.1‰	–12.3‰
RSF 009	181304	3	RP4	0.7‰	–10.4‰
RSF 010	181476	4	RP4	–0.6‰	–10.4‰
RSF 011	181403	4	Lm1 or Lm2	0.1‰	–12.0‰
RSF 012	181357	4	LP4	3.3‰	–9.4‰
RSF 013	181764	5	Lp4	2.8‰	–9.8‰
RSF 014	181723	5	LP4	–0.5‰	–10.0‰
RSF 015	181866	5	LP4	–1.1‰	–11.2‰
RSF 017	182040	6	RP4	6.3‰	–10.5‰
RSF 018	182058	6	RP4	2.3‰	–10.3‰
RSF 019	182088	7	Lp4	3.2‰	–10.3‰
RSF 020	182086	7	LP4	–1.1‰	–9.3‰
RSF 021	182159	7	Rp4	–3.1‰	–8.1%

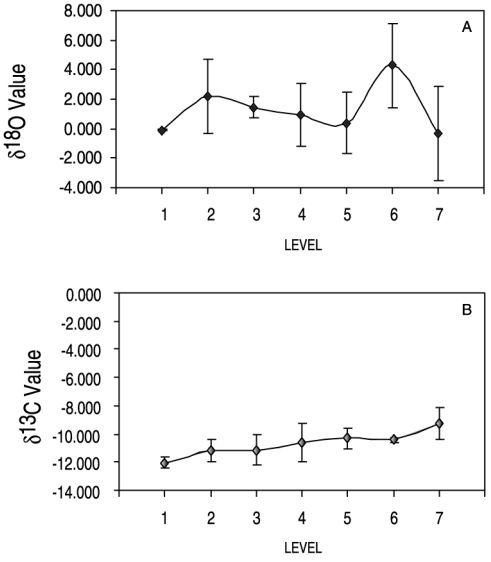

FIGURE 24.2 Oxygen (**A**) and carbon (**B**) isotope values obtained from the different levels within the Pit. Error bars represent one standard deviation from the mean.

HSD tests showed no significant differences in mean oxygen isotope values between levels.

The means for stable carbon isotope values for each level are as follows: level 1, –12.0‰, level 2, –11.2‰; level 3, –11.1‰; level 4, –10.6‰; level 5, –10.3‰; level 6, –10.4‰; level 7, –9.2‰ (figure 24.2). ANOVA suggested no significant differences between levels, but the Tukey HSD test showed a significant difference in the carbon isotope values between level 1 and level 7.

Statistical Analysis between Glacial Stages and Interglacial Stages

Mean stable oxygen isotope values for the glacial and interglacial stages are as follows: interglacial levels 1–3, 1.1‰; glacial levels 4 and 5, 0.7‰; interglacial levels 6 and 7, 1.5‰ (figure 24.3). ANOVA and Tukey HSD tests showed no significant differences in the oxygen isotope values between the glacial and interglacial stages.

Mean stable carbon isotope values for the glacial and interglacial stages are as follows: interglacial levels 1–3, –11.5‰; glacial levels 4 and 5, –10.5‰; interglacial levels 6 and 7, –9.7‰ (figure 24.3). ANOVA suggested a significant difference in carbon isotope values between the glacial and interglacial stages ($p < 0.01$). The Tukey HSD test showed a significant

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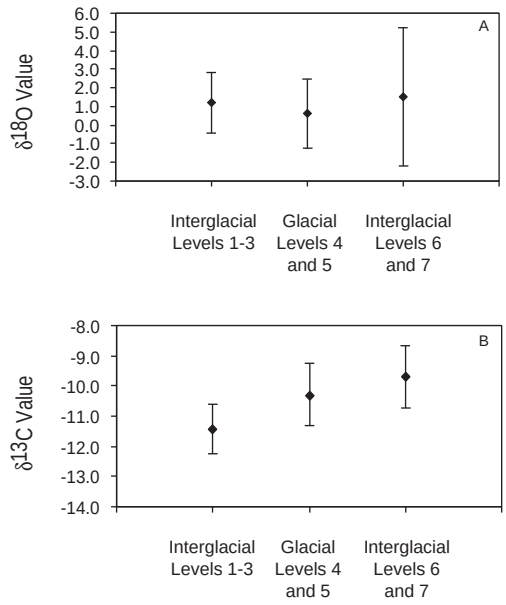

FIGURE 24.3 Oxygen (**A**) and carbon (**B**) isotope values from glacial or interglacial strata within the Pit. Error bars represent one standard deviation from the mean.

difference in carbon isotope values between interglacial levels 1–3 and interglacial levels 6 and 7.

Discussion

Possible influences on the isotopic value of marmot tooth enamel might include changes in the isotopic value of source waters, individual metabolic differences, and percentage of water obtained from plant material. Changes in the isotopic value of precipitation have been noted to occur in the Rocky Mountain region, and this observation suggests that the dominant source of precipitation for this area arises from either the Pacific Ocean or the Gulf of Mexico, depending on atmospheric circulation, which changed between glacials and interglacials (Amundson et al., 1996).

It is impossible to correlate the *Marmota* enamel $\delta^{18}O$ values with the marine oxygen isotope record because no significant differences were observed in the oxygen isotope values between either stratigraphic levels or glacial-interglacial stages. Rogers and Wang (2002) used isotopic data from *Thomomys* and *Geomys* (pocket gophers) incisors to examine the correlation of similarly aged deposits at Hansen Bluff, Colorado and SAM Cave, northern New Mexico, with the marine oxygen isotope record. Although direct correlation with the marine record was not possible with the Porcupine Cave *Marmota* data, it is of interest that the general trends shown in figure 24.2—highest $\delta^{18}O$ values in level 6, little change in levels 5–2, and a slight drop in level 1—would not contradict the pattern noted for the pocket gopher teeth from Hansen Bluff–SAM Cave for the interval 780–900 Ka.

Assuming small sample size is not the issue, a possible reason for the constancy of oxygen isotope values through all levels and glacial-interglacial stages might be that there was no significant shift in the meteoric water isotope values during the time sampled. Another possibility is that marmots' life history, specifically hibernation, may influence the isotopic value of their tooth enamel. Munson (1984) has shown that the eruption of the fully formed fourth premolar and third molar of marmots from eastern North America occurs in May, soon after the animals emerge from hibernation. This finding would suggest that these teeth were forming and mineralizing late in the hibernation period. The isotopic values displayed by the teeth may not therefore represent values related to differences in the isotopic value of meteoric water, since body water probably equilibrated to a different value from that of meteoric water ingested before hibernation. The isotopic signal may therefore be overprinted by differences in metabolism, body temperature, or both among individuals during hibernation.

Using adult teeth, as described in the Materials and Methods section, avoided the problems that would be introduced by comparing juveniles and adults. However, given the small sample sizes, it is impossible to know whether the apparent isotopic values vary between levels as a result of comparing males to females, large individuals to smaller ones, or pregnant females to nonpregnant individuals. Such gender and size differences could conceivably have an effect on the metabolism and ultimately the isotopic value of tooth enamel. If an individual marmot obtained a significant amount of water from plant leaves, seeds, or fruits, it might display different enamel isotope values than an individual that drank a significant amount (Sponheimer and Lee-Thorp, 1999, 2001; Helliker and Ehleringer, 2000).

The $\delta^{13}C$ values displayed by *Marmota* teeth at all stratigraphic levels suggest a C_3-dominated diet because mean values at all levels were more negative than –8.0‰. This result is consistent with the modern flora around Porcupine Cave, with a flora growing at 2900 m elevation elsewhere (Sage et al., 1999), and with expected conditions in the Pleistocene. The significant differences observed between levels 1 and 7, or between interglacial levels 1–3 and interglacial levels 6 and 7, may indicate a gradual shift to a flora with a more positive C_3 photosynthetic pathway, a shift to a more open habitat where CO_2 was not recycled, or inconsistent local CO_2 levels in the atmosphere, which would cause photosynthetic changes in the flora. Without further knowledge of the flora over the time period studied, through study of either macro- or microbotanical data, an unambiguous choice between these alternative explanations is impossible. Other possibilities that would cause a significant shift in $\delta^{13}C$ values are summarized by Ehleringer et al. (1991), Ehleringer and Monson (1993), Heaton (1999), Sage and Monson (1999), and references therein. The carbon isotope values in *Marmota* tooth enamel, similar to the oxygen isotope values, show no correlation with glacial-interglacial cycles per se. However, the carbon shift does parallel the overall climatic drying trend in the sequence and is compatible with the Hansen Bluff–SAM Cave data for the time between 780 and 900 Ka.

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Conclusions

Oxygen isotope analysis of tooth enamel in *Marmota* showed no significant difference between levels within the Pit, or between glacial strata and interglacial strata. Therefore it was impossible to correlate the $\delta^{18}O$ values from fossil marmot enamel with the marine oxygen isotope record. Hibernation characterized by a lower metabolism and little ingestion of water for most of the year may introduce metabolic effects that mask any isotopic differences that in other taxa might hold a climate signal.

Analysis of $\delta^{13}C$ values suggests that the marmots from Porcupine Cave were feeding on a C_3 diet. This is consistent with the modern flora growing around Porcupine cave, and generally with flora expected at the cave's current elevation. The $\delta^{13}C$ values do not appear to correlate with any glacial-interglacial cycling. Significant differences between levels 1 and 7, and between interglacial levels 1–3 and interglacial levels 6 and 7, suggest a change in the dominant flora over the time period studied. At the same time, other evidence indicates an overall drying trend (Barnosky et al., chapter 23). The $\delta^{13}C$ values show no cycling or apparent correlation with the marine carbon isotope record.

Although it was impossible to correlate strata within the Pit with the marine isotope record using *Marmota* tooth enamel isotope values, the technique may still have potential for this site. The significance of the isotopic stability at the site requires further analysis focusing on animals that are obligate drinkers and do not hibernate, or perhaps by microsampling marmot incisors, in which a hibernation line is visible and would facilitate sampling parts of the tooth that were growing when the animal was not hibernating (Goodwin and Gonzalez, 2001).

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