Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

ORIGINAL RESEARCH

Aging cheetahs using gum-line recession and evaluation of expert-based aging techniques

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Keywords
Acinonyx jubatus; carnivore age estimation; felids; gingival recession; gum recession.

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Editor: Andrew Kitchener
Associate Editor: Isabel Barja

Received 4 November 2023; revised 7 March 2025; accepted 21 March 2025

doi:10.1111/jzo.70017

Abstract

Accurate aging is a useful tool in wildlife management, providing critical information for population dynamics research, age-specific limiting factors, and conservation efforts. Many methods used to age mammalian carnivores are either invasive, expensive, or inconvenient to use in the field. In felids, the gum-line recession has been found to accurately estimate the age of female mountain lions and tigers. In contrast, expert-based aging techniques used on cheetahs (*Acinonyx jubatus*) can only categorize adults into broad age classes. We assessed whether the gum-line recession of the upper canine teeth provides reliable information for aging cheetahs by using measurements on cheetahs of known age ($n = 37$) in sex-specific linear models. We found a significant positive relationship between gum-line recession and known age for both female ($n = 21$) and male cheetahs ($n = 16$), and we contribute herein sex-specific regression models that can be used to age the animals based on the gum-line measurements collected during handling. In addition, we compared expert-derived age categories assigned to cheetahs of unknown age ($n = 23$) to the ages estimated by the gum-line recession models. Expert-based aging produced similar results to gum-line recession for some individuals, but the ages of other individuals were underestimated by biologists, particularly for older cheetahs. Our data show that gum-line recession measurements provide biologists with a reliable, minimally invasive, and convenient technique to age cheetahs in the field, and we encourage validation of this technique in other field-intensive projects involving felid capture.

Introduction

Accurate aging of wildlife individuals is an essential tool in wildlife management. For example, age can be integrated into population dynamics research for age-structured population models (Abadi et al., 2010) to investigate time-series trajectories and age-specific limiting factors. Such models have particular applied value for managing populations that are threatened, conservation-reliant, or economically important. For example, understanding which age class individuals are more likely to get into conflict with people and the underlying sociobiology for the respective species can help decisions related to harvest vs. alternative management strategies (i.e., puma [*Puma concolor*]: Teichman, Cristescu & Darimont, 2016). In addition, accurate age can be useful for regulating trophy hunting for minimizing impacts on long-term population persistence (i.e., leopard [*Panthera pardus*]: Balme, Hunter & Braczkowski, 2012; lion [*Panthera leo*]: Lindsey et al., 2013; White et al., 2016). In some jurisdictions, legislation only allows animals of a certain age to be taken from the wild (i.e., lion: White et al., 2016) and being able to understand age-specific survival is essential to guide management activities. Accurate age estimation is especially important for large carnivores because they typically exist at low population densities and face ongoing population declines in many regions, making age-specific conservation measures crucial for their management and survival (Estes et al., 2011; Ripple et al., 2014).

There are a variety of methods used to age carnivores, and they can be broadly classified into non-invasive versus requiring animal handling. Non-invasive methods rely on observing the individual either visually or through camera traps and require interpretation of phenotype. For example, African lions and leopards can be aged through features such as mane characteristics, facial scarring, nose pigmentation, ear condition, or dewlap size (Balme et al., 2012; Miller et al., 2016). Animal handling enables close inspection of skull and dental characteristics and may involve cranial measurements (Gay &

Best, 1996), pulp cavity to tooth area comparison (White et al., 2016), or counting cementum annuli from a tooth that is extracted from dead specimens or from live individuals under anesthesia (Calvert & Ramsay, 1998; Goodwin & Ballard, 1985). However, some of these methods are particularly invasive, require expensive equipment, and are not feasible in the field (Peers et al., 2021).

Gum-line recession (GLR) of the upper canines has promise to be a cost-effective and accurate method to age carnivores without the need for tooth extraction (Peers et al., 2021). GLR has been evaluated and found to be effective at estimating the age of female mountain lions (Laundré et al., 2000) and tigers (*Panthera tigris*) (Fàbregas & Garcés-Narro, 2014) but was ineffective for aging Canada lynx (*Lynx canadensis*) (Peers et al., 2021), suggesting that its applicability may vary across species. For African felids, such as leopards, tooth wear has been used for age estimation, but not GLR (Stander, 1997). Cheetah age has traditionally been estimated by using a combination of phenotypic characteristics including weight, height, coat patterns, tooth wear, and color (Marker & Dickman, 2003). While these methods are non-invasive, they have only been able to classify adult cheetahs into broad 4-year age categories, limiting their precision and thus utility in age-structured models. For example, Schmidt et al. (2019) used radiography to demonstrate correlations between age and various cranial features, such as the closure of the coronal suture, the maximum length of the frontal sinus, the condylobasal length, hard palate, and facial length. They concluded that when used in conjunction with the pulp size of the upper canine, these values can be used to approximate the age of cheetahs. However, due to the radiographic equipment required, this method is rarely practical in field settings involving anesthesia of free-ranging cheetah individuals. While there is no evaluation to compare the current cheetah-aging techniques, aging methods will offer different challenges, as they can be expensive (i.e., radiographs), imprecise (i.e., generalist body measurements), or invasive (i.e., tooth extraction for enumeration of cementum annuli). GLR offers a potential alternative that requires minimal equipment and can be applied during routine animal processing, but it is essential to further investigate its utility compared to these established methods. While GLR has proven successful in some species, the variability of results across felids emphasizes the need for species-specific validation, making this study an important first step in evaluating its effectiveness for cheetahs.

Herein, we use information on known age cheetahs in combination with upper canine GLR data on the same individuals to generate separate regression models for aging female and male cheetahs based on GLR measurements. We then apply the model to individuals of unknown birth dates to estimate their age in months and to test the following hypotheses: (1) expert-based aging based on visual interpretation of phenotypic characteristics (Marker & Dickman, 2003) would provide accurate age estimates and (2) that there would be concordance between expert-estimated and GLR-modeled aging of cheetah individuals. To our knowledge, this is the first attempt to use GLR to age African felids.

Materials and methods

Study individuals

We conducted the study on southern African cheetahs (*Acinonyx jubatus jubatus*) that were handled at the Cheetah Conservation Fund in Namibia (hereafter, CCF). These Namibian cheetahs are brought to the CCF center as part of perceived or real human-wildlife conflict, typically originating from live captures by farmers on livestock farms, or sometimes from voluntary surrenders or confiscations of individuals that were kept as pets. Animals go through a health screening and sample collection procedure at CCF's veterinary clinic. Cheetahs remain at CCF for varying periods of time, with releasable individuals being returned to the wild, non-releasable cheetahs staying in captivity, and wild-born cheetah cubs going through a rehabilitation program to prepare them for release in the wild (Walker et al., 2022). While in captivity, cheetahs are housed in large enclosures (1 hectare per animal) at CCF, surrounded by predator-proof fencing and living in groups of three to six animals. They are fed a diet consisting of 1.5–2 kg of horse (*Equus* caballus) or donkey (*Equus asinus*) meat and occasionally wild ungulates, such as impalas (*Aepyceros melampus*), oryx (*Oryx gazella*) and zebras (*Equus quagga*), for 6 days per week and having one fasting day. The frequency of feeding wild ungulates varies, but it typically occurs once a month, depending on availability. The meat portions provided include pieces of bone from the legs, spine, or ribs, as well as meat slabs without any bones. The skin from the horse, donkey, and wild ungulates is generally removed during feeding. Viscera is not a regular component of their diet, with only heart and liver being given in small quantities. It is not uncommon for animals to hunt guinea fowls (*Numida meleagris*) that enter the enclosures. All animals receive nutritional supplements in order to better support their needs (Predator Supplement, PetX, Pretoria, South Africa). None of the individuals participating in this study had a history of oral or dental diseases and, to the best of our knowledge, none was seen chewing on anthropogenic structures. The diet in captivity likely has minimal effect on GLR and thus likely aligns with GLR of wild animals, whereas it may affect tooth wear, another aging technique, and tooth wear of captive animals may thus not reflect wild cheetah age.

Data collection

Cheetah upper canine GLR measurements on live animals were recorded during a 10-year period (February 2012 to December 2021). Cheetahs were anesthetized at CCF's veterinary clinic or in the field with mostly three different drug combinations over the years (ketamine [Novecy pharmacy, 19 Eros, Namibia, 3.5 mg/kg IM] combined with medetomidine [Novecy pharmacy, 19 Eros, Namibia, 0.035 mg/kg IM] and butorphanol [Novecy pharmacy, 19 Eros, Namibia, 0.2 mg/kg IM]; ketamine 4–5 mg/kg combined with medetomidine 0.03 mg/kg and midazolam [Pfizer, Kalamazoo, MI, US, 0.2–0.6 mg/kg IM], and in the initial years, telazol [Zoetis Inc, Kalamazoo, MI, US, 2–7 mg/kg, IM]). Anesthesia was maintained with either propofol (Pfizer, Kalamazoo, MI, US, 4–10 mg/kg IV bolus), ketamine (1.5 mg/kg IM), or inhalation anesthesia with isoflurane (Frenisus Kabi, Toronto, ON, Canada) when intubated. Chemical immobilization occurred for routine check-ups or in response to acute medical issues, such as traumas, snake bites, and internal diseases such as glomeruloscleroses and gastritis, never only for GLR measurements. GLR was measured by two individuals, one of whom trained the other and generally maintained close supervision. The measurement was taken at the frontal side of the upper canines from the gum tissue to the visible gum line on the upper canine (Currier, 1979) (Fig. 1) using a digital Mitutoyo Digimatic Caliper (error ± 0.002 cm). Depending on the work-up, measurements were only taken on one upper canine or on both. We used GLR measurements on the upper right canine for analyses because fewer measurements were available for the upper left canine and to eliminate noise in the data from averaging values of both upper canines. Only cheetahs above 12 months old were included in the study, as cheetahs typically develop all of their adult teeth by 1 year of age (Marker & Dickman, 2003).

Cheetahs that arrived at CCF as cubs were typically mobile, dependent on their mothers, but orphaned. They were assigned an age to the nearest ± 3 months, based on the expert assessment that considered cub weight, tooth eruption patterns, and known growth curves (Basto et al., n.d.). These cheetahs were classified as 'known age' individuals for the purpose of this study, and their GLR was measured once they reached adulthood. Depending on the individual, GLR was measured during radio-collaring immediately prior to release to the wild; once they were in the wild and captured for deployment of a new radiocollar, or to attend to medical emergencies; or for failed release candidates, measured during their long-term captivity period. Cheetahs that arrived at CCF as adults were classified as 'unknown age' with regard to their GLR-based aging, but their GLR was measured, and they were assigned to age categories according to expert-based criteria laid out in Marker and Dickman (2003).

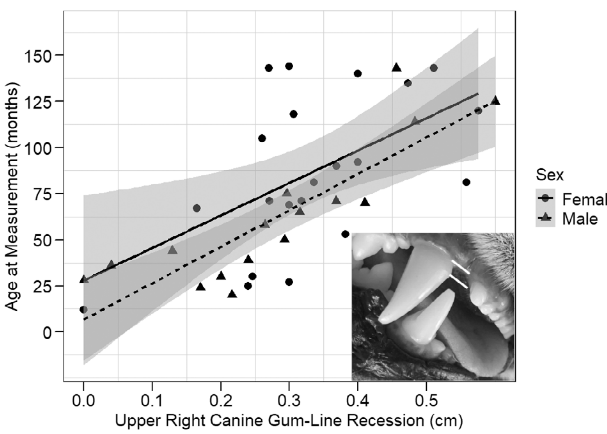

Figure 1 Regression analysis of age (in months) based on the gum-line recession (in cm) of the upper right canine in cheetahs of known age, with data presented separately for females ($n = 21$) and males ($n = 16$). The inset photo illustrates how gum-line recession is measured on the upper canine of a cheetah.

Data analysis

We performed simple linear regression separately for females ($n = 21$) and males ($n = 16$), of known age (i.e., which arrived as small cubs), where age in months was the dependent variable and GLR the predictor. Only a few individuals had multiple measurements of GLR, but to avoid pseudoreplication by including multiple measurements for the same individual, we randomly picked one measurement per animal for inclusion in the regression models. The fit of regression models was assessed using the coefficient of determination (R^2) and associated probability values (Seber & Lee, 2003).

When cheetahs of unknown age arrived at CCF, they were aged visually based on Marker and Dickman (2003). We adapted five age categories for cheetahs other than cubs: Subadult (12–29 months), Young Adult (30–47 months), Prime Adult (48–95 months), Old Adult (96–143 months), and Very Old Adult (144+ months) (Marker & Dickman, 2003). For each of these categorized individuals that also had GLR measured, we used the sex-specific parameter estimates from the regression models on known age animals to predict the respective age based on GLR. We then binned the GLR model-derived ages in the five categories adapted from Marker and Dickman (2003). We compared the frequency of age distribution in each category between expert-based and GLR-modeled outputs using sex-specific Fisher's Exact tests.

We carried out the statistical and graphing procedures in R Studio v.2021.09.0 + 351 (R Core Team, 2021), using base R functions and packages *ggplot2* (Wickham, 2016), *patchwork* (Pedersen, 2023), and *ggthemes* (Arnold, 2021).

Results

Gum-line recession in animals with known age

We were able to include a total of 37 cheetahs (21 females and 16 males) of known age in the regression modeling (Table S1). Linear regression of age in months according to upper right canine GLR showed significant positive slopes for females ($\beta_{female} = 176.04$, 95% CI = 46.81–305.27, $p = 0.010$, $n = 21$, $R^2 = 0.30$) and males ($\beta_{male} = 197.46$, 95% CI = 127.90–267.02, $p = 2.8\text{e-}05$, $n = 16$, $R^2 = 0.73$) (Fig. 1).

We obtained the following regression equations that can be used to predict the age in months of cheetahs based on the GLR of the upper right canine:

$$\text{Females : Age(months)} = 176.04 \times \text{upper right canine GLR(cm)} + 28.00$$

$$\text{Males : Age(months)} = 197.46 \times \text{upper right canine GLR(cm)} + 6.71$$

Analysis of concordance between expert opinion and GLR-derived aging in animals with unknown age

Twenty-three cheetahs of unknown age were available to include in the analysis on concordance between expert and upper right canine GLR-derived aging. The overall differences in classification between expert-based and GLR-modeled age categories were not statistically significant for male ($p = 0.186$, $n = 14$) or female cheetahs ($p = 0.536$, $n = 9$). The age categorization was particularly similar between the two methods for cheetah individuals with GLR ≤ 0.4 cm (Fig. 2).

However, for individuals of both sexes with GLR >0.4 cm, expert classification generally appeared to yield younger animals than model-derived estimates based on GLR (Figs 2 and 3).

Discussion

Using sex-specific regression, we were able to derive equations for aging cheetahs based on GLR. To our knowledge, this is the first time this is achieved for felids in Africa. The GLR equations presented in this study are particularly robust due to their development based on animals with known ages, specifically cheetahs that arrived as cubs. Given that these cubs were still nursing, that we relied on tooth eruption patterns, cub growth curves from daily observations, and on expert inspection of the cubs (Basto et al., n.d.), any potential error in age estimation is biologically insignificant, with a maximum deviation of only a few months. This high level of accuracy underscores the strength of the GLR method as an effective tool for age estimation. While some differences in tooth wear due to diet variability may occur in wild cheetahs, the GLR method remains highly comparable and offers a valuable resource for researchers in both captive and wild settings.

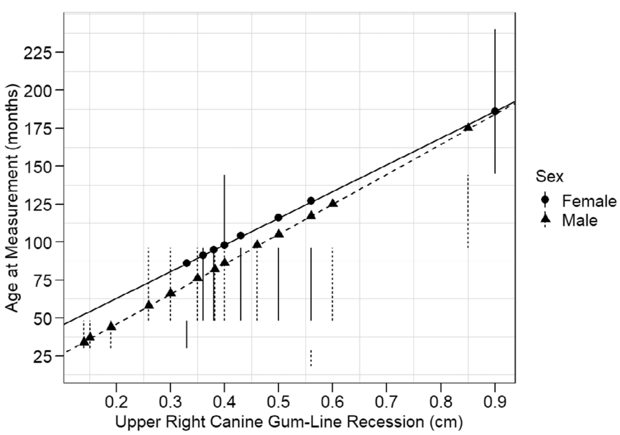

Figure 2 Comparison of GLR modeled and expert opinion-derived age categories for cheetahs with unknown ages. GLR-modeled ages are represented as data points, while expert opinion age categories are marked by vertical lines based on ranges from Marker and Dickman (2003) ($n_{female} = 9$, $n_{male} = 14$). Some data points overlap due to similar upper right canine GLR measurements among individuals.

Due to the logistics of large carnivore research, especially the difficulty of longitudinal studies from the cub phase to adulthood, the core of the study is based on a relatively small sample size of known-age cheetah individuals, which does not necessarily capture all variation. However, this approach is the most robust method for cheetahs to date, given that no other objective measures exist. Our sample sizes, the use of cheetahs of known age to parameterize aging equations, and also sex-specific differentiation of aging estimates advance previous GLR studies on felids.

Our study also revealed differences in the age classification of cheetahs between GLR models and expert-based assessments. Although there was concordance in age classification between the methods for some individuals, expert-based age estimates occasionally estimated cheetah ages to be lower than the ones derived by the model created with animals of known ages.

Most studies have estimated carnivore age based on GLR pool sexes, possibly due to sample size limitations or an inherent assumption that both sexes would have comparable rates of GLR. Indeed, Laundré et al. (2000), Fàbregas and Garcés-Narro (2014), and Peers et al. (2021), the latter with a large sample size, pooled the data across males and females. Our sample size was sufficient to partition the data according to sex, and we chose to perform sex-specific regression because canine length in carnivores differs between the sexes as a result of sexual dimorphism (Gittleman & Valkenburgh, 1997). It could therefore be expected that the gum-line recession might differ as well among sexes. In addition, male felids are more likely to hunt larger prey, while females are typically smaller and may have different hunting and feeding strategies (Cristescu et al., 2022; Radloff & Du Toit, 2004). The influence on GLR of routine capturing and handling of large prey that may be dangerous and may take a long time to subdue, as well as a potentially large volume of flesh (vs. bone and skin) available for consumption, and resulting potential differences in gum-line wear among male and female carnivores, have not been quantified. However, carrying out distinct analyses by sex allows for some of this variability to be modeled.

Two data points for 0 cm GLR were included in the models. These points include one male that showed no recession at 28 months and one female at 12 months of age. We included these data in the models to account for the variation around the age at which GLR begins to occur. Adult dentition of cheetahs is completely developed by 12 months (Marker & Dickman, 2003); GLR could theoretically begin once the teeth have fully erupted.

The hypothesis that expert-based aging, based on visual assessment of cheetah individuals, would yield similar results to classification based on the quantitative GLR method, was supported for both females and males, as no significant differences were observed. However, many of the expert-based age estimates (depicted by vertical lines in Fig. 2) are below the regression lines, indicating that expert-derived aging yielded younger ages for some cheetahs compared to GLR-modeled outputs (Fig. 3). For example, the expert-derived assignments did not classify any males as ‘Very Old Adult’, whereas based

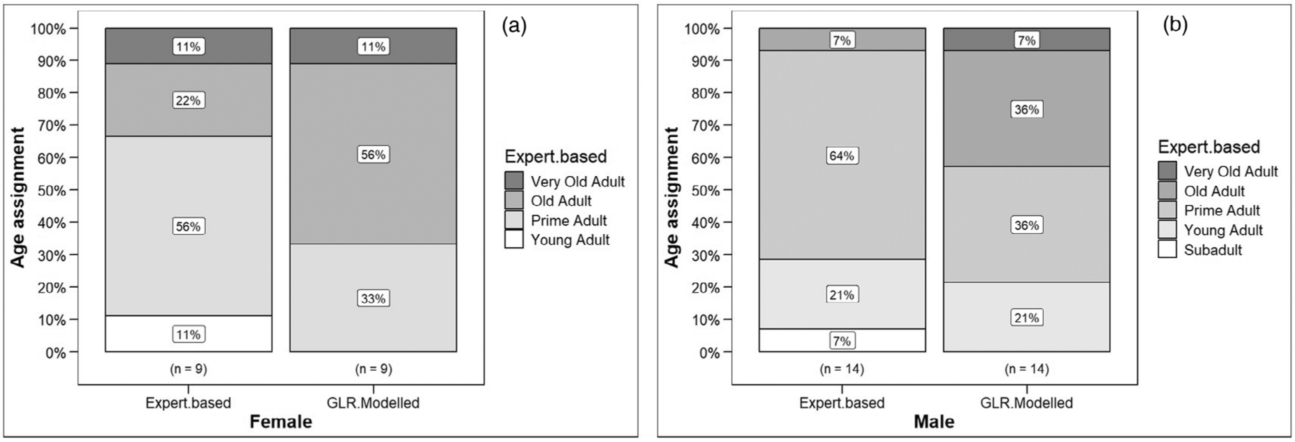

Figure 3 Comparison of percentage of cheetahs of unknown age assigned to different age classes using the GLR model versus expert-based assessment. Data is presented for females (a) and males (b) cheetahs ($n_{female} = 9$, $n_{male} = 14$). Age classes are adapted from Marker and Dickman (2003): Subadult (12–29 months), Young Adult (30–47 months), Prime Adult (48–95 months), Old Adult (96–143 months), and Very Old Adult (144+ months).

on GLR one male was determined to be close to 15 years of age, an exceptionally old age for cheetahs. In contrast, expert estimation classified one female as 'Young Adult' and one male as 'Subadult', which are categories not assigned by the GLR models to the respective sexes (Table S2).

Captive diets that consist of ground meat and that lack bone, skin, or connective tissue have been found to have negative effects on dental health, as ground meat does not fulfill the mechanical aspect of handling a carcass and can lead to an increase in periodontal disease and dental calculus accumulation (Kapoor et al., 2016). The resident cheetahs at CCF were fed pieces of meat attached to bone, mimicking a diet in the wild; however, the absence of skin, as well as the exclusion of viscera, might possibly influence the GLR and is an area of future investigation. The GLR method for aging is not recommended for use on carcasses or harvested individuals because gums may recede further as carcasses dry (Laundré et al., 2000). In addition, pulp cavity width and cementum annuli are likely more accurate and appropriate, as these specimens are not alive.

For carnivores that are handled through live capture, age has often been estimated based on tooth wear and condition (Delahay et al., 2011; Marti & Ryser-Degiorgis, 2018; Olifiers et al., 2010; Stander, 1997) and sometimes indexed by body size measurements (Olifiers et al., 2010). In some studies, a tooth is extracted for cementum annuli analysis (Bodkin et al., 1997; Cattet et al., 2008) given that this type of analysis has greater estimation precision than aging based on tooth wear (Gipson et al., 2000). Calculations of pulp cavity-to-tooth width ratios have also been used to estimate carnivore age with mixed success (Kershaw et al., 2005; Knowlton & Whittemore, 2001). In comparison, GLR has been used less frequently despite its potential utility and minimal invasiveness.

When live captures of carnivores are carried out, measuring GLR is straightforward with digital calipers and can provide a cost-effective, more accurate, and simple way for ecologists and wildlife managers to age the study animals following basic training. Measurements can then be used in conjunction with parameter estimates from regression equations, such as those contributed by this study for cheetahs. We recommend the expansion of the GLR approach for aging other species of carnivores due to its ease of use and replicability, and because it minimizes subjectivity among observers that characterizes some expert-based assessments.

Acknowledgements

We thank Becky Johnston, Eli Walker, Anne Schmidt-Küntzel, and veterinarians at the Cheetah Conservation Fund for assistance with handling and processing cheetahs. The research was performed under the research permit numbers AN2018051701 and AN202101032 of the Cheetah Conservation Fund (Namibian-based Institute RCIV0001). We are grateful to the Editor, Liaan Minnie, and an anonymous reviewer for constructive feedback that greatly improved the manuscript.

Author contributions

BC and AFB conceived the ideas and designed the methodology; LM collected the data; BC, ML, and AFB analyzed the data; BC, AFB, and ML wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

The data and R code for this work are archived at Zenodo (https://zenodo.org/records/10.5281/zenodo.10068430).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Sex, age and gum-line recession of the upper right canine of known age cheetah individuals included in regression modelling of cheetah age based on gum-line recession.

Table S2. Sex and gum-line recession of the upper right canine of cheetah individuals of unknown age for which age was estimated comparatively based on expert inspection and modeled gum-line recession.