10-1-2006

Lower Incisors of Prairie Dogs (Cynomys) as Biorecorders of Hibernation and Season of Death

H. Thomas Goodwin
Andrews University, goodwin@andrews.edu

Eva M. Ryckman
Andrews University, ryeva@andrews.edu

LOWER INCISORS OF PRAIRIE DOGS (*CYNOMYS*) AS BIORECORDERS OF HIBERNATION AND SEASON OF DEATH

H. Thomas Goodwin* and Eva M. Ryckman

Biology Department, Andrews University, Berrien Springs, MI 49104, USA
Present address of EMR: School of Medicine, Loma Linda University, Loma Linda, CA 92350, USA

Incremental dentin and associated enamel, features visible at the surface of lower incisors of rodents, may chronicle important life-history information. This study investigated surface features of lower incisors representing 4 taxa of prairie dogs (*Cynomys*) in relation to hibernation and season of year. A set of abnormalities in dentin and enamel, observed on 20 of 138 incisors, always chronicled an event ending in late winter or early spring and was interpreted as a hibernation mark. Hibernation was recorded in the incisors of obligate hibernators, *C. leucurus* and *C. gunnisoni*, as well as in 20% of specimens of the facultative heterotherm, *C. ludovicianus*, with relevant winter growth record. Inspection of prairie dog incisors from museum collections elucidated patterns in the timing of spring emergence across species, sexes, and elevations. Growth rates of prairie dog incisors (estimated from thicknesses of circadian dentin increments) showed general seasonal patterns when pooled by sex and species, but daily growth rates recorded along individual incisors often fluctuated idiosyncratically through time. However, incisors that chronicled a significant temporal trend in daily growth rate registered either early-season (increased growth rate through time) or late-season growth (decreased growth rate through time). None of 11 late-Pleistocene fossil *C. niobrarius churcherii* exhibited a hibernation mark, but 4 of these exhibited significant decrease in growth rate along their incisors, probably indicating animals that died late in the active season.

Key words: *Cynomys gunnisoni*, *Cynomys leucurus*, *Cynomys ludovicianus*, *Cynomys niobrarius churcherii*, dentin, enamel, growth rates, hibernation, seasonality

Prairie dogs (*Cynomys*) show striking divergence in over-winter strategies. Two species in the subgenus *Cynomys* (Mexican prairie dog [*C. mexicanus*] and black-tailed prairie dog [*C. ludovicianus*]) have been observed to be active year-round (Fitzgerald et al. 1994; Tileston and Lechleitner 1966; Treviño-Villarreal 1990), suggesting that these 2 species do not hibernate under typical field conditions. However, laboratory animals of *C. ludovicianus* stressed with cold and lack of food display periods of relatively shallow, facultative torpor during winter (Harlow and Menkens 1986). Free-ranging animals likewise enter periodic, shallow torpor during winter in 3 colonies of *C. ludovicianus* from northern Colorado (Lehmer and Biggins 2005; Lehmer et al. 2001), but not in 1 colony of *C. ludovicianus* previously studied from the same region (Bakko et al. 1988). Moreover, *C. ludovicianus* from 1 colony

in Colorado reportedly exhibit profiles of body temperature that are characteristic of hibernation, with extended bouts of deep torpor punctuated by periodic arousals (unpublished data cited by Lehmer and Biggins [2005]). Thus, although many individuals of *C. ludovicianus* do not hibernate over winter, the species is capable of facultative torpor and perhaps hibernation during winter.

In contrast, 3 species in the subgenus *Leucocrossuromys* (Gunnison's prairie dog [*C. gunnisoni*], white-tailed prairie dog [*C. leucurus*], and Utah prairie dog [*C. parvidens*]) appear to be obligate hibernators under field conditions; colonies typically cease aboveground activity for several months over winter, resuming activity in late winter or early spring (e.g., Bakko and Brown 1967; Clark 1977; Fitzgerald and Lechleitner 1974; Holdenried and Morlan 1956). Additionally, captive animals of *C. gunnisoni* (Rayor et al. 1987) and *C. leucurus* (Bakko and Nahorniak 1986) display winter body-temperature profiles characteristic of hibernation, with extended bouts of deep torpor punctuated by periodic arousals. Free-ranging individuals of *C. parvidens* exhibit the same pattern (Lehmer and Biggins 2005).

* Correspondent: goodwin@andrews.edu

For prairie dogs that hibernate, timing of vernal emergence varies by sex, year, elevation, and species. Multiyear studies of *C. leucurus* from colonies on the Laramie Plains in Wyoming reported 1st emergence between early February and early March, depending on year, with males emerging 2–3 weeks before females (Bakko and Brown 1967; Clark 1977). Radiotelemetric study of *C. parvidens* in Utah demonstrated mean arousal from the final torpor bout of hibernation on 16 February at a low-elevation colony (1,575 m), but on 17 March at a high-elevation colony (Lehmer and Biggins 2005). Studies of *C. gunnisoni* have reported 1st emergence ranging from early March at ~1,750–1,950 m elevation near Santa Fe, New Mexico (Holdenried and Morlan 1956) to mid-April at ~2,850 m elevation in South Park, Colorado (Fitzgerald and Lechleitner 1974). Temporal trends in emergence dates have not been reported for *Cynomys*, but records of 1st spring sighting of a related ground-dwelling squirrel, *Marmota flaviventris* (yellow-bellied marmot), near Gothic, Colorado, from 1976 to 1999 indicate significantly earlier emergence through time, tracking regional climate warming (Inouye et al. 2000). Thus, emergence dates might serve as a proxy for species' response to climate change.

The duration of hibernation is likewise variable, although individual prairie dogs typically spend multiple months sequestered below ground with much of this time spent in torpor. For example, depending on elevation, free-ranging *C. parvidens* averaged ~4–5.5 months in hibernation (time between 1st and last torpor bouts) and ~2,600–4,280 h during hibernation in torpor (Lehmer and Biggins 2005). Individual adults of *C. leucurus* on the Laramie Plains of Wyoming reportedly cease aboveground activity for ~7 months (Clark 1977), although the proportion of this time spent in torpor is unknown.

The profound, extended metabolic depression of hibernation leaves a diagnostic hibernation mark in lower incisors of other ground-dwelling squirrels, including yellow-bellied marmots (Rinaldi 1999) and 3 species of ground squirrels (Wyoming ground squirrel [*Spermophilus elegans*], Richardson's ground squirrel [*S. richardsonii*], and arctic ground squirrel [*S. parryii*]—Goodwin et al. 2005). This mark is evident in both enamel and dentin of the incisor's surface because of the mechanism of rodent incisor growth and the way such growth is expressed at the tooth's surface.

Rodent incisors are evergrowing, with both enamel and dentin deposited throughout life at the incisor's base. Dentin is laid down in cone-shaped increments along the pulp cavity, with the "lip" of each increment expressed topographically at the tooth's surface as a ridge–valley couplet extending transversely across the tooth (Fig. 1A). These increments normally display circadian periodicity in rodents (Klevezal 1996; Schour and Steadman 1935), a result confirmed for yellow-bellied marmots (Rinaldi 1999) and ground squirrels (Goodwin et al. 2005). Enamel is likewise laid down at the base of the incisor, but it is draped over the external, labial surface of the growing tooth. As a result, enamel caps the labial surface of the tooth, hiding incremental dentin labial to the enamel–dentin junction (Fig. 1B).

During hibernation, dentin deposition rates drop precipitously (to ~2.5% of normal rates in *Spermophilus*—Goodwin et al. 2005), and the resultant dentin usually exhibits either very narrow increments without circadian periodicity or homogeneous structure without visible increments (Goodwin et al. 2005; Rinaldi 1999). Enamel deposition is likewise affected, with hibernation enamel often exhibiting surface irregularities and, most characteristically, a thickened sleeve clearly demarcated from prehibernation enamel (Goodwin et al. 2005). The combination of these features is what forms a reliable hibernation mark.

Additionally, for museum specimens with known day of death, the day on which hibernation ended can be inferred from the incisor by counting daily dentin increments between the hibernation mark and base of tooth, which records the last growth increment before death. However, because incisors are evergrowing, and each increment of basal growth must be matched by equivalent wear at the apex of the tooth, any feature of the tooth's structure, including the hibernation mark, will be preserved along the tooth only for a limited time. In *Spermophilus*, an incisor typically preserves a record of the last 7–11.5 weeks of life (Goodwin et al. 2005).

In addition to recording hibernation, incremental dentin also preserves daily longitudinal growth rates during the time of its deposition. Rinaldi and Cole (2004) documented a primary seasonal signal in growth rates across a sample of beaver upper incisors from Kansas, with peak rates in August followed by marked decline through late summer and early autumn, a trend curiously absent from lower incisors. This signal was clear in the overall sample and in the growth records of some but not all individual upper incisors. On a finer scale, Goodwin and others (2005) showed that a reduction in dentin growth rates often correlates with parturition in female Richardson's ground squirrels. Thus, incisor growth-rate histories may provide information about seasonality and life history beyond the occurrence and timing of hibernation.

The present study investigated lower incisors of prairie dogs as potential recorders of hibernation and seasonality. Our study included 2 obligate hibernators (*C. leucurus* and *C. gunnisoni*) and a facultative heterotherm (*C. ludovicianus*) within *Cynomys*, as well as a late-Pleistocene taxon, *C. niobrarius churcherii*. We characterized surface expression of normal incisor dentin and enamel in prairie dogs and documented, characterized, and tested the reliability of a putative hibernation mark. In addition, we investigated longitudinal growth patterns in dentin of lower incisors as potential indicators of season of death. Finally, we assessed whether hibernation and season of death were recorded in lower incisors of a small sample of fossil prairie dogs.

MATERIALS AND METHODS

We surveyed large series of *C. ludovicianus*, *C. gunnisoni*, and *C. leucurus* and located specimens with at least 1 loose lower incisor (*n* = 138; Appendix I). Using low-angle, reflected incandescent light, we carefully inspected the enamel, incremental dentin, and enamel–dentin junction of 1 lower incisor per specimen under a stereomicroscope to characterize normal structure as well as look for abnormalities similar to hibernation marks in *Marmota* (Rinaldi 1999) and *Spermophilus* (Goodwin et al. 2005). Our inspection focused on the medial surface of each incisor but often also included the labial,
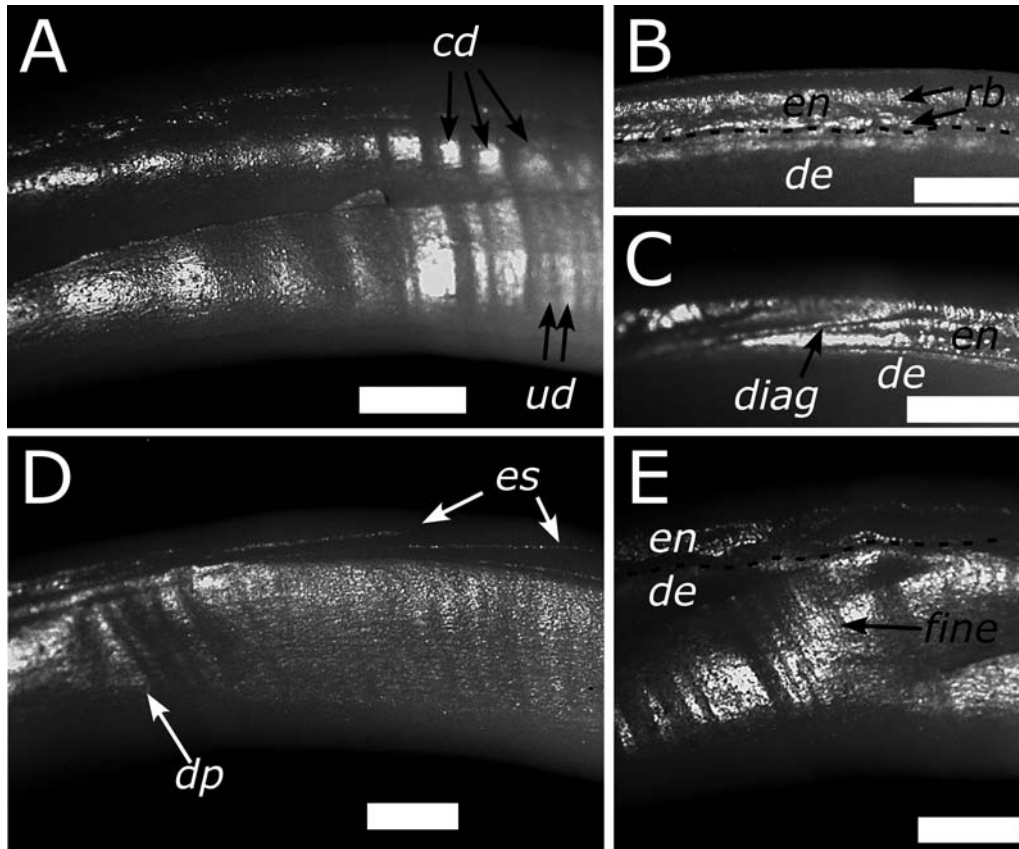
Fig. 1.—Medial surface of lower prairie dog incisors with tooth apex to the right, labial surface to the top in each. A) Incremental dentin of lower incisor. Sequence of 3 circadian increments (cd) marked with upper arrows; 1 pair of probable ultradian (frequency > 1 per day) increments (ud) marked with lower arrows. Note labial fusion of ultradian increments to form a single circadian increment. B) Normal surface appearance of enamel (en) expressed as 2 reflective bands (rb) parallel to the contact with dentin (de). Enamel–dentin junction marked with dashed line. C) Diagonal feature (diag) in surface structure of enamel associated with a hibernation mark that sometimes was evident D) as 1 or 2 enamel "sleeves" (es). Depression (dp) of medial dentin surface associated with hibernation mark also depicted in D). E) Chaotic surface topography of enamel, irregularities at the enamel–dentin junction (dashed line), and very fine dentin increments associated with the hibernation mark in many specimens. Taxa and specimens represented: *Cynomys ludovicianus* (KU 127014—A), *C. leucurus* (KU 127011—B, E), and *C. gunnisoni* (KU 126853—C, D). Scale bars represent 1 mm.

lingual, and lateral surfaces, especially on specimens that were difficult to interpret. We were purposefully blind to date of death when inspecting for these abnormalities (with a few accidental exceptions) to avoid biasing determinations given seasonal timing of hibernation. However, we subsequently obtained date of death for each specimen to facilitate further analyses.

We estimated total and (for specimens with a putative hibernation mark) posthibernation duration of record for each incisor with adequately visible increments by counting number of daily increments expressed on the medial surface. A pair of ultradian increments was sometimes superimposed on a daily increment, as was also observed in *Spermophilus*, but these could usually be distinguished from daily increments because they were less prominent and tended to fuse near the enamel–dentin junction (Fig. 1A; Goodwin et al. 2005). When recognized, ultradian increments were excluded from increment counts.

We counted dentin increments using 2 protocols. In protocol 1, as a 1st estimate, we quickly counted increments along the length of each tooth 2 or 3 times during initial inspection and took the average value rounded to the nearest increment. In protocol 2, in a subsequent analysis, we counted increments more precisely on a subset of specimens (8 *C. gunnisoni*, 34 *C. leucurus*, and 26 *C. ludovicianus*) by recording exact number of increments per marked segment (see

below), quantitatively estimating number of increments in short segments of dentin that were difficult to visualize (based on increments per millimeter in adjacent dentin), and then summing increments across marked segments to get increment total per tooth (or posthibernation). Total increment counts differed significantly between protocols applied to the same teeth (protocol 1 < protocol 2 by ~3 increments; paired Student's *t*-test: $t = 4.29$, *d.f.* = 39, $P < 0.001$), thus we only used counts obtained with protocol 2 in statistical comparisons. However, counts obtained in protocol 1 were used to plot the range of dates spanned by an incisor's increment sequence if not superceded by a count obtained using protocol 2.

We calculated a series of longitudinal dentin growth rates along incisors with adequate record. First, we lightly marked off segments of ~10 dentin increments on the medial surface using a china marking pencil, after which we counted exact number of circadian increments per segment. Second, we digitally photographed each marked incisor with a ruler in view, calibrated the scale of each image to the included ruler, and then calculated a longitudinal growth rate per segment as millimeters per day (using circadian increments as proxy for days). Marks were then removed with a moistened cotton-tipped applicator. Growth rates were not calculated for segments with estimated but not actual increment counts.
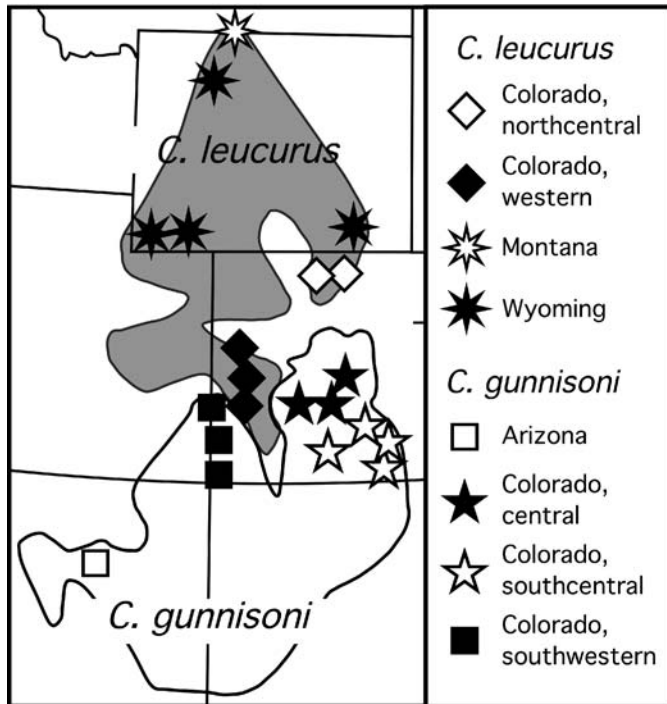
FIG. 2.—Specimen localities for *Cynomys gunnisoni* and *C. leucurus* on a base map with state boundaries and geographic ranges for the 2 species. Each geographic cluster of localities shares a common symbol to facilitate comparison with Fig. 3.

Each growth-rate value along an incisor was assigned to the median date of the segment from which it was calculated. When possible, this was expressed as day of year, calculated from known date of death (recorded at the base of the incisor) by subtracting the number of circadian increments between death and the given segment's median increment. Day of year could not be calculated for dentin increments laid down during or before a hibernation mark, because circadian periodicity of growth increments is abandoned during hibernation. For incisors with a hibernation mark, we also expressed the median date of each segment in relative terms, as number of days pre- or posthibernation (again determined by increment counts relative to a given event, in this case the hibernation mark). These values could not be expressed in absolute terms, because dates of entry into and emergence from hibernation were not known.

We performed 3 analyses to investigate temporal pattern in incisor dentin growth rates. First, we calculated average daily growth rate, separately for each month, across males and females of each species, to obtain overall seasonal variation for each sex–species category. In this analysis, each growth-rate measurement (obtained from 1 segment of increments; see above) was categorized in the month incorporating its median date. Second, we documented growth patterns along each individual incisor by plotting growth rates against day of year, or against number of days pre- or posthibernation (anchored to the beginning and end of the hibernation mark, respectively). Third, we used correlation analysis (Pearson's correlation coefficient) to identify specimens exhibiting a significant, progressive trend in daily growth rate through time, as documented along the incisor's length. For specimens with a hibernation mark, correlations were calculated separately for pre- and posthibernation sections of the tooth.

Finally, we inspected a small sample of late-Pleistocene incisors ($n = 11$; Appendix I) as a test case for documenting hibernation and season of death in fossil prairie dogs. We counted dentin increments as described in protocol 2 above, and growth-rate series were determined, plotted, and statistically analyzed as described for modern specimens. All fossils represented *C. niobrarius churcherii*, which was initially described by Burns and McGillivray (1989) as a new species from southern Canada but later subsumed within *C. niobrarius*, a widespread late-Pleistocene prairie dog of the subgenus *Leucocrossuromys* (Goodwin 1995b). Specimens came from the Courtney (West), Schowalter, and Winter sites in southern Alberta (Burns 1996).

RESULTS

Qualitative characterization of normal lower incisor surface features.—Incisor dentin of all *Cynomys* taxa that we examined exhibited surface increments interpreted as circadian in periodicity, expressed as a series of ridge–valley couplets (Fig. 1A) that extended transversely across the tooth from the enamel–dentin junction. Increments were difficult to distinguish in short segments of some teeth and were generally difficult to see on a few other teeth. The elevated ridge of a circadian increment often bore a pair of yet finer increments that, when present, typically fused near the enamel–dentin junction (Fig. 1A), a pattern also common in *Spermophilus* (Goodwin et al. 2005). These finer increments were interpreted as ultradian in periodicity and were excluded from circadian increment counts.

The enamel–dentin junction was abrupt on typical specimens (Fig. 1B). The enamel just labial to the junction exhibited a characteristic appearance under low-angle, reflected light, evident as 2 (sometimes 3) reflective bands parallel to the long axis of the tooth (Fig. 1B). These bands appear to correspond to subtle, parallel ridges in surface enamel. Enamel near the base of the incisor, which was often poorly mineralized, did not always exhibit these bands.

Qualitative characterization and distribution of putative hibernation mark.—Twenty of 138 Recent specimens (9 of 39 *C. gunnisoni*, 9 of 57 *C. leucurus*, 2 of 41 *C. ludovicianus*, and 0 of 1 *Cynomys* uncertain species) exhibited abnormalities of enamel and dentin that resembled features of a confirmed hibernation mark in *Spermophilus* (Goodwin et al. 2005). Across all Recent specimens with the putative hibernation mark, the most diagnostic attribute was a diagonal feature interrupting the continuity of enamel reflective bands labial to the enamel–dentin junction (19 of 20 specimens; Fig. 1C), sometimes expressed as a prominent enamel "sleeve" (3 of 20; Fig. 1D). This diagonal feature was doubled in 4 specimens (Fig. 1D). Correlated features of the putative hibernation mark included irregularities of enamel basal to this diagonal feature (lingual deflection of the enamel–dentin junction [15 of 20; Fig. 1E], thickening of 1 or both reflective bands sometimes causing the bands to fuse [14 of 20], and chaotic surface topography of enamel [11 of 20; Fig. 1E]), and the presence of a zone of dentin with indistinct or fine increments (17 of 20; Fig. 1E), often marked with a depression on the medial surface of the tooth (15 of 20; Fig. 1D). Seventeen of 20 specimens recorded as having the putative hibernation mark exhibited 4–6 of the attributes described above.

Six additional specimens (3 of *C. gunnisoni* [139157, 139159, and 139160] and 3 of *C. leucurus* [126938, 127967,
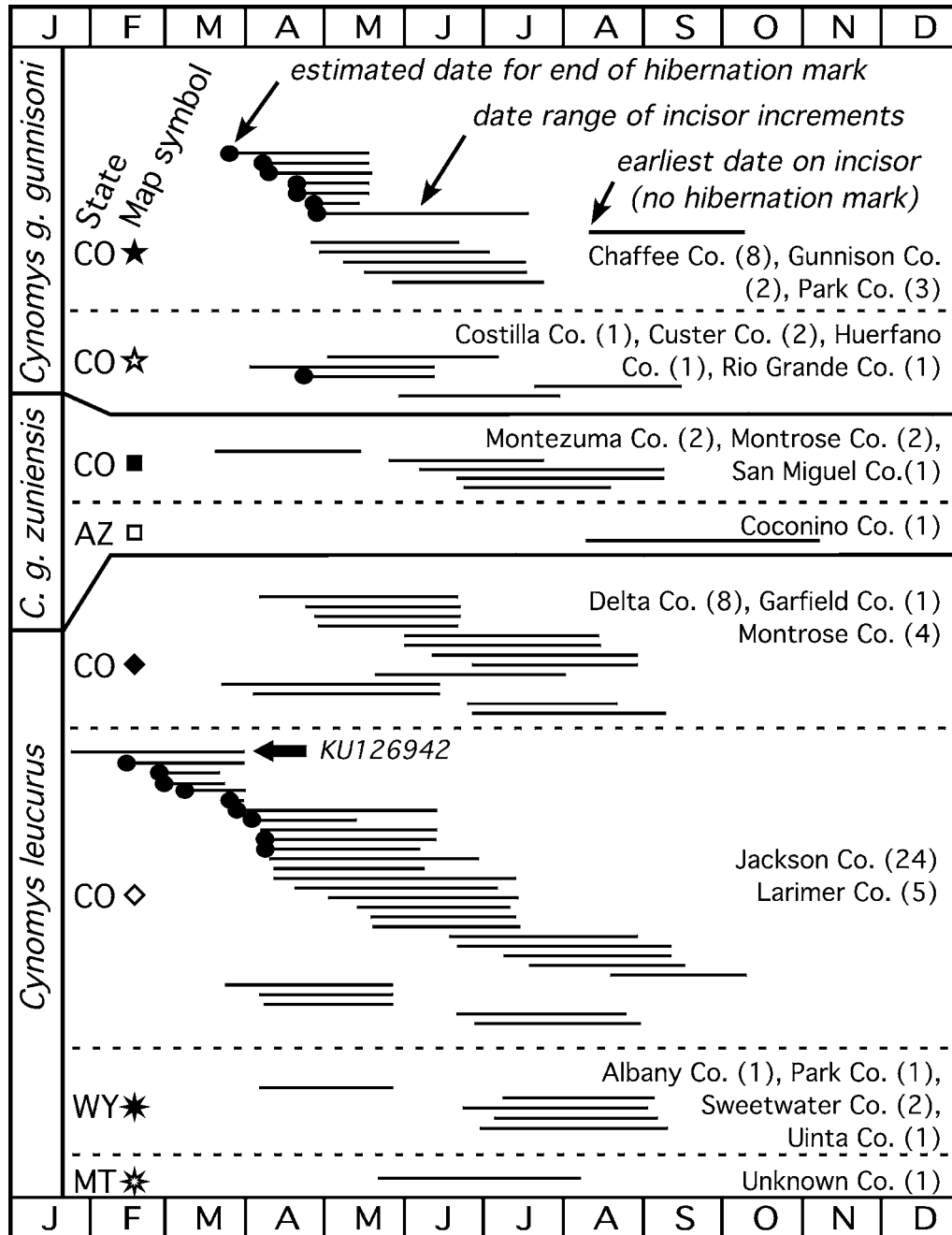
FIG. 3.—Range of dates recorded in the incremental dentin of individual incisors of *Cynomys gunnisoni* and *C. leucurus* anchored to known day of death (right end of each date range). Specimens are ordered by taxon, state (AZ = Arizona; CO = Colorado; MT = Montana; WY = Wyoming), geographic area within state (each geographic area given a separate map symbol; see Fig. 2), alphabetically by county (Co.), then by date of the earliest increment on the tooth, or date on which hibernation ended (if hibernation mark was evident, it is depicted with dark circle). Parenthetical value (*n*) after each county name indicates number of specimens.

and 136347]) exhibited dentin and enamel irregularities that somewhat resembled a hibernation mark, but all 6 lacked the diagonal feature of enamel and were recorded as not exhibiting the mark. In addition, 3 specimens of *C. leucurus* (127907, 127908, and 127917) that died as laboratory animals exhibited highly aberrant incisors with a series of abnormalities along the tooth. Nothing analogous was observed in field-caught specimens, and we suspect that laboratory conditions were responsible for the abnormalities.

The termination of the putative hibernation marks ranged from 25 March to 26 April for *C. gunnisoni* and 13 February to 6 April for *C. leucurus*, both obligate hibernators in the wild. Across geographic localities (Fig. 2), the mark was never observed in incisors of these species with a dental record postdating late April (Fig. 3). In contrast, incisor anomalies interpreted as nonhibernation features reflected events later in the active season (3 specimens of *C. leucurus* [early June–mid-August]), or could not be placed in time
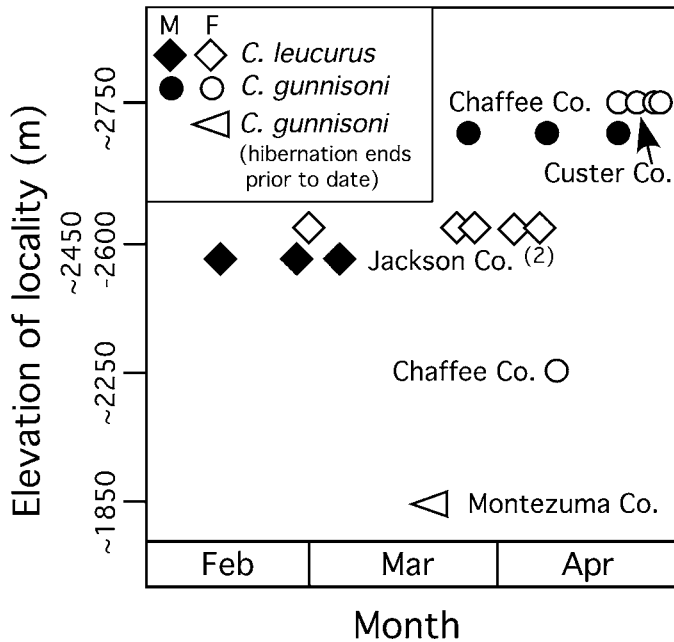
FIG. 4.—Date of end of hibernation for individual prairie dogs as a function of elevation, sex, and species. Dates are inferred by counting dentin increments from tooth base to base of hibernation mark (or apex of tooth if no hibernation mark present). All samples are from Colorado; county (Co.) names indicate location of specimens. Specimen from Montezuma County did not preserve a hibernation mark despite a record extending as early as mid-March, implying that it emerged earlier in the season. M = male; F = female.

(3 specimens of *C. gunnisoni*, because of unknown date of death).

The putative hibernation mark ended ~26–28 January in 2 specimens of the facultative heterotherm, *C. ludovicianus*. Both specimens were from Larimer County, Colorado, but were collected in different years (1959 and 1960). The mark was better developed in one of these specimens (KU 127104), with an incisor that displayed 4 of the correlated features of a hibernation mark. Other specimens of *C. ludovicianus* with incremental record extending through some or all of December–February (*n* = 8) lacked the putative hibernation mark.

The putative hibernation mark was present on incisors of males (*n* = 8) and females (*n* = 12), indicating that female-specific stressors such as parturition or lactation were not its cause. In males the hibernation mark terminated, on average, 16 days before marks in females in *C. gunnisoni* from a site in Chaffee County, Colorado, and 31 days before females in *C. leucurus* from Jackson County, Colorado (Fig. 4). Estimated emergence dates for female *C. gunnisoni* from 3 elevations in Colorado yielded a biologically sensible trend, with progressively later spring emergence at higher elevations (Fig. 4). One female *C. leucurus* (KU 126942) exhibited irregularities in dentin structure about 5 increments from the incisor base (located in time ~25 March) but showed none of the diagnostic features of the putative hibernation mark, despite an incremental record apparently extending back to 23 January (Fig. 3). It probably represented a false negative—a specimen that hibernated without leaving a diagnostic mark.

Taken together, these observations implicate hibernation as the sole cause of the putative hibernation mark in *Cynomys*, as previously reported for *Spermophilus* (Goodwin et al. 2005). Occurrence of 1 probable false negative, however, suggests that it is possible for an occasional prairie dog to go through hibernation without leaving a diagnostic surface mark.

Variation in incremental dentin by species, sex, and season.—Pooled across Recent species, lower incisors averaged 69.9 increments per tooth (*SD* = 11.4, *n* = 66), with a range of 41–93 increments per tooth. Only 2 specimens had <50 increments, and 2 additional specimens exhibited >90 increments. Thus, most incisors recorded the final 50–90 days (~7–12 weeks) of dental growth before the prairie dog's death. There was no difference in increment count per tooth across species (*C. gunnisoni*—$\bar{X}$ = 75.8, *SD* = 10.5, *n* = 8; *C. leucurus*—$\bar{X}$ = 69.2, *SD* = 10.7, *n* = 32; *C. ludovicianus*—$\bar{X}$ = 68.9, *SD* = 12.2, *n* = 26; *F* = 1.23, *d.f.* = 2, *P* > 0.05) or sexes (males—$\bar{X}$ = 70.3, *SD* = 11.0, *n* = 24; females—$\bar{X}$ = 69.0, *SD* = 11.2, *n* = 41; *t* = 0.45, *d.f.* = 63, *P* > 0.05).

Average daily growth rate of incisors (average width of daily increments per tooth) varied significantly across species (*F* = 8.64, *d.f.* = 2, *P* < 0.001) with incisors of *C. ludovicianus* having significantly wider daily increments ($\bar{X}$ = 0.55 mm, *SD* = 0.12, *n* = 26) than those of *C. leucurus* ($\bar{X}$ = 0.48 mm, *SD* = 0.07, *n* = 33) and *C. gunnisoni* ($\bar{X}$ = 0.41 mm, *SD* = 0.07, *n* = 8); incisors of *C. leucurus* and *C. gunnisoni* were not significantly different from each other on this variable.

Average daily dentin growth rates grouped by species, sex, and month displayed several general seasonal trends (Figs. 5A–C). Male and female *C. gunnisoni* (Fig. 5A) and *C. leucurus* (Fig. 5B) showed sequential increase in growth rates across the first 3 months posthibernation with highest rates in midspring to summer. Both sexes of *C. ludovicianus* showed elevated growth rates in May–July, males also showed elevated growth rates in February–March, and both sexes exhibited progressive decrease in average growth rates from July to December (Fig. 5C).

Growth series for individual incisors, plotted against date of growth, were characteristically idiosyncratic, even when we controlled for time and place of collection (Figs. 5D and 5E). However, some overlapping growth series did seem to covary (e.g., specimens 126926 and 126927; Fig. 5D). Growth series anchored to the hibernation mark (plotted as days pre- and posthibernation) showed a greater tendency to covary, notably prehibernation reduction in growth of *C. leucurus* (Fig. 6A, top panel) and posthibernation increase in growth of *C. gunnisoni* (Fig. 6A, bottom panel).

Most incisors showed no significant trend in growth rates through time. Five incisors exhibited a significant increase in growth rate through time (*P* < 0.05), and in all 5 the growth series represented spring to midsummer growth (ending in May in *C. gunnisoni*, June in *C. leucurus*, and June or July in *C. ludovicianus*). Seven other incisors exhibited a significant decrease in growth rate through time (*P* < 0.05); these incisors typically recorded late-season growth that ended in July, August, or September in *C. leucurus* (adults ceased above-ground activity in July and August, with juveniles following
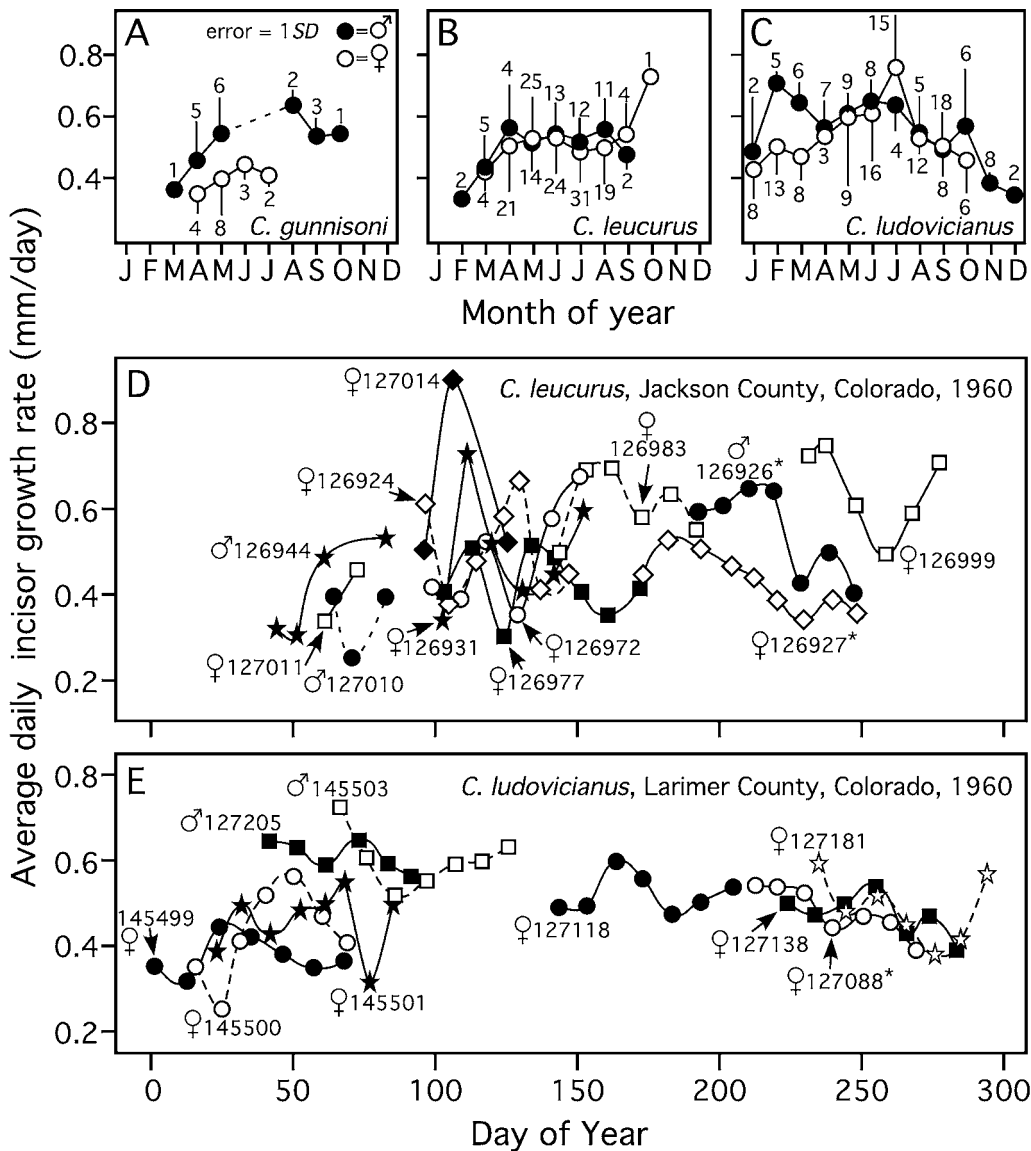
FIG. 5.—Temporal variation in incisor growth rates of prairie dogs. A–C) Average daily growth rates given separately for males and females, with each growth-rate measurement (taken from a marked segment of increments; see "Materials and Methods") categorized by month in which the segment's median date falls. Number of growth-rate measurements per sample is indicated. D, E) Longitudinal growth series for individual incisors, plotted against day of year, from 2 discrete samples that control for taxon, geographic location (adjacent counties in northern Colorado), and year of collection (all from 1960).

soon thereafter, in a population from Wyoming studied by Clark [1977]), and September, October, or November in *C. ludovicianus*. Finally, 5 of 7 incisors with a hibernation mark and sufficient prehibernation record to evaluate growth trends through time showed significant decrease in growth rates through time leading up to hibernation ($P < 0.05$; Fig. 6A, both panels). Taken together, these observations suggest general seasonal trends in growth despite the idiosyncratic variation common to individuals.

Hibernation and season of death in fossil prairie dogs.— None of 11 late-Pleistocene *C. niobrarius churcherii* from southern Alberta exhibited a hibernation mark. However, 4 of 10 specimens with measured growth series showed a signifi- cant, negative association with time (Fig. 7, top panel; $P <$

0.05 for 2 specimens, $P < 0.001$ for 2 additional specimens). This probably indicates death either late in the active season, or perhaps during hibernation but before the diagnostic hiberna- tion mark had formed. The remaining 6 specimens with measured increments show no association between growth and time (Fig. 7, bottom panel). Several of these show high volatility in growth rates (e.g., P87.8.2 and P86.3.230). Season of death is unknown for these specimens.

DISCUSSION

Incremental dentin and labial enamel in *Cynomys* is very similar to that in *Spermophilus* (Goodwin et al. 2005). Both display high variability in increment width and distinctness,
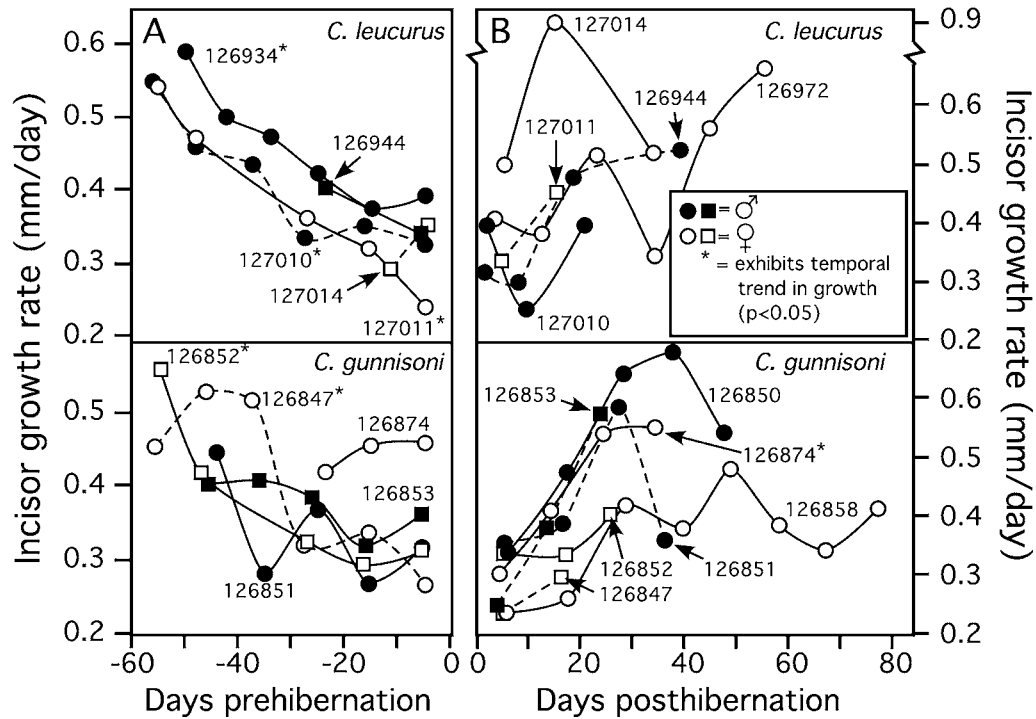
Fig. 6.—Longitudinal growth series for individual incisors, plotted as days before or after the hibernation mark. A) Prehibernation growth series for *Cynomys leucurus* (top panel) and *C. gunnisoni* (bottom panel). B) Posthibernation growth series for *C. leucurus* (top panel) and *C. gunnisoni* (bottom panel). Incisors displaying a significant temporal trend in growth rates are marked with an asterisk (*).

commonly exhibit a pair of ultradian increments superimposed on the more prominent features that we interpret to be daily in frequency (Fig. 1A), and exhibit a characteristic enamel surface feature visible as 2 (sometimes 3 in *Cynomys*) reflective bands adjacent and parallel to the enamel–dentin junction (Fig. 1B). We were unable to directly confirm the circadian interpretation of major dentin increments with the specimens of *Cynomys* available to us, but the strong similarity to increments in *Spermophilus* with confirmed circadian periodicity provides indirect confirmation (Goodwin et al. 2005). Dentin increments on the surface of yellow-bellied marmot incisors also exhibit circadian periodicity (Rinaldi 1999).

The hibernation mark in *Cynomys* likewise resembles a mark described for *Spermophilus*. The most diagnostic feature in both taxa is a diagonal discontinuity in enamel structure labial to the enamel–dentin junction (Fig. 1B), although this feature forms a thickened enamel sleeve more commonly in *Spermophilus* (51 of 58 specimens; Goodwin et al. 2005) than in *Cynomys* (3 of 20 specimens; Fig. 1D). Other features of the hibernation mark shared with *Spermophilus* include deflection at the enamel–dentin junction, irregularities in surface topography of enamel, depression of the dentin surface adjacent to malformed enamel, and presence of indistinct or very fine increments within the mark.

Taxonomic, sex, and temporal distributions of the putative hibernation mark strongly implicate hibernation as its cause. We observed no false positives for the hibernation mark (expression of a mark outside late winter and early spring), but 1 specimen of the obligate hibernator, *C. leucurus* (KU 126942; Fig. 3), may represent a "false negative" (failure to

express the mark when expected despite an incremental record back to ~23 January). All other individuals of obligate hibernating species (*C. leucurus* and *C. gunnisoni*) that lacked a hibernation mark died sufficiently late in the active season to have lost the mark through growth and attrition of the incisor.

For the facultative heterotherm, *C. ludovicianus*, presence of a hibernation mark in 20% of incisors (2 of 10) with relevant winter growth record probably indicates that these individuals hibernated, a result consistent with reported hibernation in 1 free-ranging colony of *C. ludovicianus* (unpublished data cited by Lehmer and Biggins [2005]). If this frequency is representative, hibernation may be more common in *C. ludovicianus* than previously recognized. Multiple, short-term, shallow bouts of torpor punctuating normothermic activity are common in free-ranging *C. ludovicianus* (Lehmer and Biggins 2005) but do not appear to be reflected individually in the winter growth record of incisors; we observed no incisor of *C. ludovicianus* with multiple disruptions attributable to such bouts.

Earliest inferred dates for end of hibernation in our study are generally consistent with published accounts from populations at similar elevations. For example, we inferred an earliest emergence date of 25 March in a small sample of *C. gunnisoni* from ~2,750 m elevation in central Colorado (Fig. 4), slightly later than typical mid-March emergence reported from ~2,500 m in Gunnison County, Colorado (Rayor 1985). The record of *C. leucurus* from Jackson County is more puzzling. All 9 specimens with a hibernation mark were collected in 1959–1960, during the time that Tileston and Lechleitner (1966) investigated a population at ~2,500 m in this county. Tileston

and Lechleitner (1966) anecdotally reported 1st emergence of males in late March with females soon thereafter but did not give specific data. In contrast, examination of our data indicates that all 3 males and 1 of 6 females ended hibernation between ~13 February and ~3 March, with remaining females emerging in late March or early April (Figs. 3 and 4). We suspect that their report is in error. Consistent with this interpretation, 1st emergence of male *C. leucurus* at ~2,200 m near Laramie, Wyoming, varied from early February to early March, depending on year (Bakko and Brown 1967; Clark 1977), but never was as late as late March.

Males typically precede females in emergence from hibernation across hibernating ground-dwelling squirrels (Michener 1984), by 2–3 weeks in *C. leucurus* (Clark 1977). It is not surprising that *C. gunnisoni* exhibits a similar pattern (Fig. 4), although this is not well documented in the literature. This undoubtedly explains much of the "gap" between clusters of early and late emergence dates in the Jackson County sample (Figs. 2 and 3), because 3 of 4 specimens in the "early" cluster are males. However, the 4.5-week difference between average male and female emergence dates seems anomalous. Perhaps male *C. leucurus* terminate hibernation several days before emerging, a pattern documented in *S. parryii* (Buck and Barnes 1999) and *S. richardsonii* (Michener 1993), which would increase the perceived lag between male and female emergence. However, we are not aware of independent evidence for this interpretation from existing life-history studies of hibernating prairie dogs. Also, such a model does not explain early emergence (~28 February) inferred for 1 female in the sample (Fig. 4).

Likewise, it is reasonable to expect early emergence in low-elevation habitats where winter terminates earlier in the year than at higher elevations (Fig. 4), a pattern documented in free-ranging *C. parvidens* (Lehmer and Biggins 2005). The ability to detect such patterns after the fact, by inspecting the growth record of incisors, should facilitate study of geographic, elevational, and temporal variation in end of hibernation based on existing museum collections, data that may be helpful in addressing conservation questions. For example, Inouye and others (2000) regressed date of 1st spring observation of *M. flaviventris* against year, across 1976–1999 at a single site in the Rocky Mountains of Colorado, demonstrating earlier emergence through time. Because spring snow cover was not reduced during this interval, marmots are now emerging through deeper snow cover than in the past. It may be possible to investigate similar trends over longer time intervals, because North American mammal collections often include specimens back to about 1900.

Incisor growth rate, inferred from widths of circadian increments, varies substantially within and among individuals as well as species (Fig. 5). Summary seasonal patterns are suggestive (Figs. 5A–C), but it is difficult to apply these to infer season of death of individual specimens. However, specimens that show a significant temporal trend in growth rates along the incisor are broadly assignable to season, with significant increase in growth rate through time characteristic of incisors that chronicle growth in spring to early summer, and
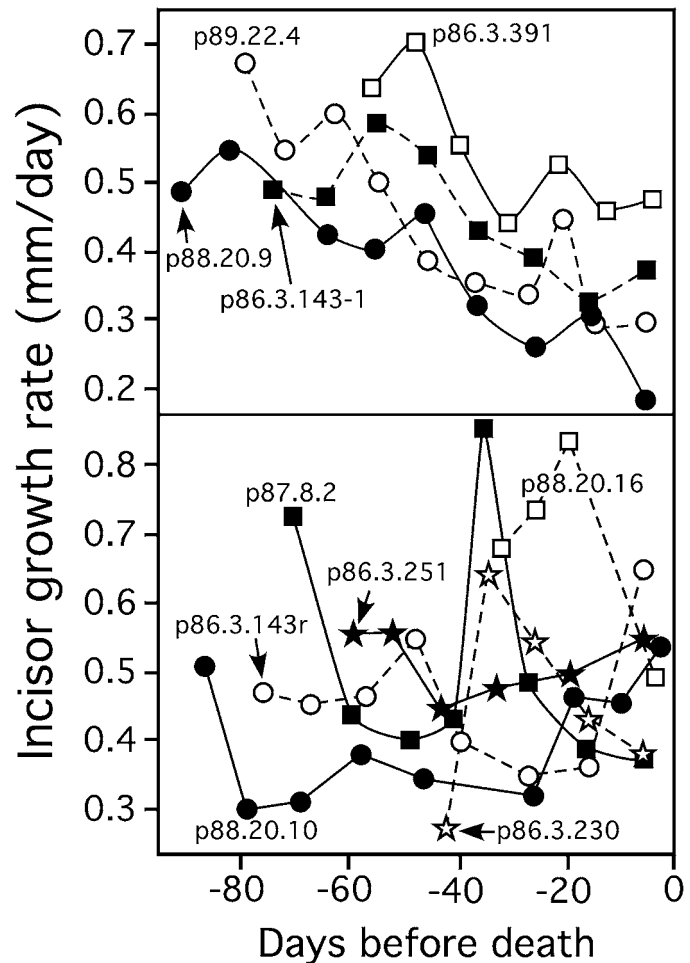
FIG. 7.—Longitudinal growth series for individual fossil incisors of *Cynomys niobrarius churcherii*, anchored to last increment before death (recorded by the incisor's most basal increment). Incisors displaying statistically significant reduction in growth rate up to time of death are displayed in the top panel; those showing no temporal trend in growth rates are displayed in the bottom panel.

significant decrease through time characteristic of specimens that record late-season growth. Particularly striking is the strong tendency for incisor growth rates to decrease through time over the 40–60 days leading up to hibernation (Fig. 6A).

None of the fossils we examined exhibited a hibernation mark, but significant decrease in growth rates over 40–60 days up to time of death indicates that 4 of these specimens probably died late in the active season, or perhaps during hibernation. We have no data on how and when the mark develops during hibernation in *Cynomys*, but it is likely that individuals who died early in hibernation would not show the mark. The mark begins forming in the 1st few months of hibernation in *S. richardsonii*, but is very weakly developed at that time (Goodwin et al. 2005). The 2 prairie dog subgenera, which today include species that differ in typical hibernation biology, commonly co-occur in late-Pleistocene sites of the central Great Plains and southern Rocky Mountains (Goodwin 1995a). Determination of the hibernation biology of these fossil prairie dogs might provide insight into how these closely

related species partitioned ecological resources during the Pleistocene.

LITERATURE CITED

BAKKO, E. B., AND L. N. BROWN. 1967. Breeding biology of the white-tailed prairie dog, *Cynomys leucurus*, in Wyoming. Journal of Mammalogy 102:100–112.

BAKKO, E. B., AND J. NAHORNIAK. 1986. Torpor patterns in captive white-tailed prairie dogs (*Cynomys leucurus*). Journal of Mammalogy 67:576–578.

BAKKO, E. B., W. P. PORTER, AND B. A. WUNDER. 1988. Body temperature patterns in black-tailed prairie dogs in the field. Canadian Journal of Zoology 66:1783–1789.

BUCK, C. L., AND B. M. BARNES. 1999. Annual cycle of body composition and hibernation in free-living arctic ground squirrels. Journal of Mammalogy 80:430–442.

BURNS, J. A. 1996. Review of Pleistocene zoogeography of prairie dogs (genus *Cynomys*) in western Canada with notes on their burrow architecture. Pp. 34–53 in Palaeoecology and palaeoenvironments of late Cenozoic mammals: tributes to the career of C. S. (Rufus) Churcher (K. M. Stewart and K. L. Seymour, eds.). University of Toronto Press and Royal Ontario Museum, Toronto, Ontario, Canada.

BURNS, J. A., AND W. B. MCGILLIVRAY. 1989. A new prairie dog, *Cynomys churcherii*, from the late Pleistocene of southern Alberta. Canadian Journal of Zoology 67:2633–2639.

CLARK, T. W. 1977. Ecology and ethology of the white-tailed prairie dog (*Cynomys leucurus*). Milwaukee Public Museum Publications in Biology and Geology 3:1–97.

FITZGERALD, J. P., AND R. R. LECHLEITNER. 1974. Observations on the biology of Gunnison's prairie dog in central Colorado. American Midland Naturalist 92:146–163.

FITZGERALD, J. P., C. A. MEANEY, AND D. M. ARMSTRONG. 1994. Mammals of Colorado. University Press of Colorado, Niwot.

GOODWIN, H. T. 1995a. Pliocene–Pleistocene biogeographic history of prairie dogs, genus *Cynomys* (Sciuridae). Journal of Mammalogy 76:100–122.

GOODWIN, H. T. 1995b. Systematic revision of fossil prairie dogs with descriptions of two new species. Miscellaneous Publications, Museum of Natural History, University of Kansas 86:1–38.

GOODWIN, H. T., G. R. MICHENER, D. GONZALEZ, AND C. E. RINALDI. 2005. Hibernation is recorded in lower incisors of Recent and fossil ground squirrels (*Spermophilus*). Journal of Mammalogy 86:323–332.

HARLOW, H. J., AND G. E. MENKENS, JR. 1986. A comparison of hibernation in the black-tailed prairie dog, white-tailed prairie dog, and Wyoming ground squirrel. Canadian Journal of Zoology 64:793–796.

HOLDENRIED, R., AND H. B. MORLAN. 1956. A field study of wild mammals and fleas of Santa Fe County, New Mexico. American Midland Naturalist 55:369–381.

INOUYE, D. W., B. BARR, K. B. ARMITAGE, AND B. D. INOUYE. 2000. Climate change is affecting altitudinal migrants and hibernating species. Proceedings of the National Academy of Sciences 97:1630–1633.

KLEVEZAL, G. A. 1996. Recording structures of mammals: determination of age and reconstruction of life history. A. A. Balkema, Rotterdam, The Netherlands.

LEHMER, E. M., AND D. E. BIGGINS. 2005. Variation in torpor patterns of free-ranging black-tailed and Utah prairie dogs across gradients of elevation. Journal of Mammalogy 86:15–21.

LEHMER, E. M., B. VAN HORNE, B. KULBARTZ, AND G. L. FLORANT. 2001. Facultative torpor in free-ranging black-tailed prairie dogs (*Cynomys ludovicianus*). Journal of Mammalogy 82:551–557.

MICHENER, G. R. 1984. Age, sex, and species differences in the annual cycles of ground-dwelling sciurids: implications for sociality. Pp. 81–107 in The biology of ground-dwelling squirrels (J. O. Murie and G. R. Michener, eds.). University of Nebraska Press, Lincoln.

MICHENER, G. R. 1993. Sexual differences in hibernaculum contents of Richardson's ground squirrels: males store food. Pp. 109–118 in Life in the cold: ecological, physiological, and molecular mechanisms (C. Carey, G. L. Florant, B. A. Wunder, and B. Horwitz, eds.). Westview Press, Boulder, Colorado.

RAYOR, L. S. 1985. Effects of habitat quality on growth, age of first reproduction, and dispersal in Gunnison's prairie dogs (*Cynomys gunnisoni*). Canadian Journal of Zoology 63:2835–2840.

RAYOR, L. S., A. K. BRODY, AND C. GILBERT. 1987. Hibernation in the Gunnison's prairie dog. Journal of Mammalogy 68:147–150.

RINALDI, C. 1999. A record of hibernation in the incisor teeth of Recent and fossil marmots (*Marmota flaviventris*). Pp. 112–119 in Proceedings of the 11th International Symposium on Dental Morphology (J. T. Mayhall and T. Heikkinen, eds.). Oulu University Press, Oulu, Finland.

RINALDI, C. E., AND T. M. COLE III. 2004. Environmental seasonality and incremental growth rates of beaver (*Castor canadensis*) incisors: implications for palaeobiology. Palaeogeography, Palaeoclimatology, Palaeoecology 206:289–301.

SCHOUR, I., AND S. R. STEADMAN. 1935. The growth pattern and daily rhythm of the incisor of the rat. Anatomical Record 63:325–333.

TILESTON, J. V., AND R. R. LECHLEITNER. 1966. Some comparisons of the black-tailed and white-tailed prairie dogs in north-central Colorado. American Midland Naturalist 75:292–316.

TREVIÑO-VILLARREAL, J. 1990. The annual cycle of the Mexican prairie dog (*Cynomys mexicanus*). Occasional Papers, Museum of Natural History, University of Kansas 139:1–27.

Submitted 19 May 2005. Accepted 21 March 2006.

Associate Editor was Craig L. Frank.

APPENDIX I

Specimens examined.—All specimens of *Cynomys ludovicianus, C. gunnisoni*, and *C. leucurus* are from the University of Kansas Museum of Natural History (KU), Lawrence, Kansas, and all fossil *C. niobrarius churcherii* are from the Provincial Museum of Alberta (PMA), Edmonton, Alberta, Canada. Specimens with a hibernation mark are indicated with an asterisk (*).

Cynomys ludovicianus ludovicianus.—Colorado: Larimer County, 127066, 127069, 127088, 127094, 127103, 127104*, 127106, 127107, 127112, 127118, 127120, 127123*, 127125, 127137, 127138, 127139, 127152, 127162, 127176, 127180, 127181, 127205, 145480, 145499, 145500, 145501, 145503; Morgan County, 127214. Kansas: Gove County, 1913; Meade County, 14014, 14098, 53075; Morton County, 5288; Pawnee County, 139148; Stafford

County, 4504, 5642. Montana: Carter County, 123277, 123278. Oklahoma: Texas County, 127923. South Dakota: Harding County, 86372, 98299.

Cynomys gunnisoni gunnisoni.—Colorado: Chaffee County, 126847*, 126850*, 126851*, 126852*, 126853*, 126857, 126858*, 126859, 126871, 126874*, 126875*; Costilla County, 126887; Custer County, 126882, 126883*; Gunnison County, 126746, 126842, 126843, 139157, 139158, 139159, 139160; Huerfano County, 126885; Park County, 126829, 126832, 126833; Rio Grande County, 149468; Saguache County, 18250.

Cynomys gunnisoni zuniensis.—Arizona: Coconino County, 121789. Colorado: Montezuma County, 123845, 126907, 126910, 127906, 145441, 145445, 145450, 145452; Montrose County, 126891, 126894; San Miguel County, 126895.

Cynomys leucurus.—Colorado: Delta County, 123898, 123900, 123901, 123902, 123903, 123904, 127038, 127041, 127046, 127047, 127908; Garfield County, 127025, 127026; Jackson County, 126923, 126924, 126925*, 126926, 126927, 126931, 126933, 126934*, 126938, 126942, 126943*, 126944*, 126950, 126952*, 126953, 126970, 126972*, 126977, 126983, 126997, 126999, 127010*, 127011*, 127014*, 127967, 145457; Larimer County, 127017, 127018, 127019, 127020, 127022, 127023; Montrose County, 123906, 123907, 127052, 127223, 127907, 127917. Montana: unreported county (from "Pryor Mountains"), 136347. Wyoming: Albany County, 126920; Park County, 21866; Sweetwater County, 16801, 16802; Uinta County, 16800.

Cynomys unidentified to species.—Colorado: Delta County, 145509.

Fossil Cynomys niobrarius churcherii.—Canada, Alberta (locality data from Burns [1996]): Courtney (West) site (26.5 km NE of Drumheller), P88.20.9, P88.20.10, P88.20.16, P88.20.32, P89.22.4; Schowalter site (30 km SE of Drumheller), P87.8.2; Winter site (28 km NE of Drumheller), P86.3.143 (2 specimens), P86.3.230, P86.3.251, P86.3.391.