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HIBERNATION IS RECORDED IN LOWER INCISORS OF RECENT AND FOSSIL GROUND SQUIRRELS (*SPERMOPHILUS*)

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Incremental dentin and associated enamel, features visible on the surface of lower incisors, were characterized for 3 species of ground squirrels (*Spermophilus*): Pleistocene and Recent *S. elegans*, Recent *S. richardsonii*, and Recent *S. parryii*. A hibernation mark was evident in incisor dentin and enamel, most characteristically as a sleeve of enamel terminating basally adjacent to medially depressed dentin with indistinct and often very fine increments. This mark was absent in juveniles but present in older animals of both sexes for at least 6 weeks after hibernation, eventually being lost through growth and wear of the incisor. Temporal association with hibernation was confirmed from specimens of *S. richardsonii* with known dates of hibernation. Parturition and onset of lactation were usually associated with reduction in thickness of dentin increments but could not be recognized unambiguously. Combining wear stage of cheek teeth with the presence and location of the hibernation mark allowed placement of many specimens into age and season categories at time of death (young of year, early-season adults, and late-season adults). Examination of lower incisors of Pleistocene *S. elegans* from Porcupine Cave in central Colorado showed that hibernation was recorded in fossils and confirmed the utility of event-anchored incremental dentin in elucidating taphonomic questions.

Key words: dentin, enamel, hibernation, *Spermophilus elegans*, *Spermophilus parryii*, *Spermophilus richardsonii*, taphonomy, tooth wear

Dentin is laid down incrementally throughout life in the evergrowing incisors of rodents and lagomorphs, with each increment forming a cone-shaped deposit along the pulp cavity (Schour and Steadman 1935). Dentin increments visible in stained incisor sections show circadian periodicity in laboratory rats (Schour and Steadman 1935), a variety of wild rodents studied in the laboratory and field (Klevezal 1996), and rabbits (Rosenberg and Simmons 1980). Other increment periodicities include ultradian (<24 h) in rats (Ohtsuka and Shinoda 1995) and both ultradian and infradian (>24 h) in rabbits (Rosenberg and Simmons 1980).

Circadian dentin increments vary in thickness across time (e.g., Rinaldi 1999), potentially reflecting stressors that influence an animal's ability to deposit dentin (Schour and Massler 1949). Stained sections of incremental incisor dentin may reveal life-history information such as lactation in female ground squirrels (*Spermophilus*) and hibernation in species of birch

mice (*Sicista*), ground squirrels, and marmots (*Marmota*—Klevezal 1996; Klevezal and Mina 1990). Because preparation of stained tooth-sections is time consuming and destroys the tooth, sectioning of teeth is of limited usefulness for deducing life-history information. Fortunately, dentin increments also are visible at the incisor surface, expressed topographically as a series of ridge–valley couplets and visible under low-angle light as reflective bands oriented transversely relative to the long axis of the incisor (Figs. 1A–C). These couplets may represent alternating hypercalcified and hypocalcified layers (Rinaldi 1999). Increment patterns may thus be documented nondestructively and in situ by inspection under low-angle light, with or without surface staining. In yellow-bellied marmots (*M. flaviventris*), surface increments were typically circadian in periodicity, but additionally they sometimes exhibited a distinctive pattern of very fine increments, often associated with abnormal enamel, that served as a hibernation mark (Rinaldi 1999). During the active season, an incisor typically preserves 2–3 months of record (see “Results”), with older increments lost to wear and growth of the tooth.

Our study investigated the surface expression of incremental dentin and associated enamel in lower incisors of 3 species of ground squirrels of the subgenus *Spermophilus* (*Spermophilus*),

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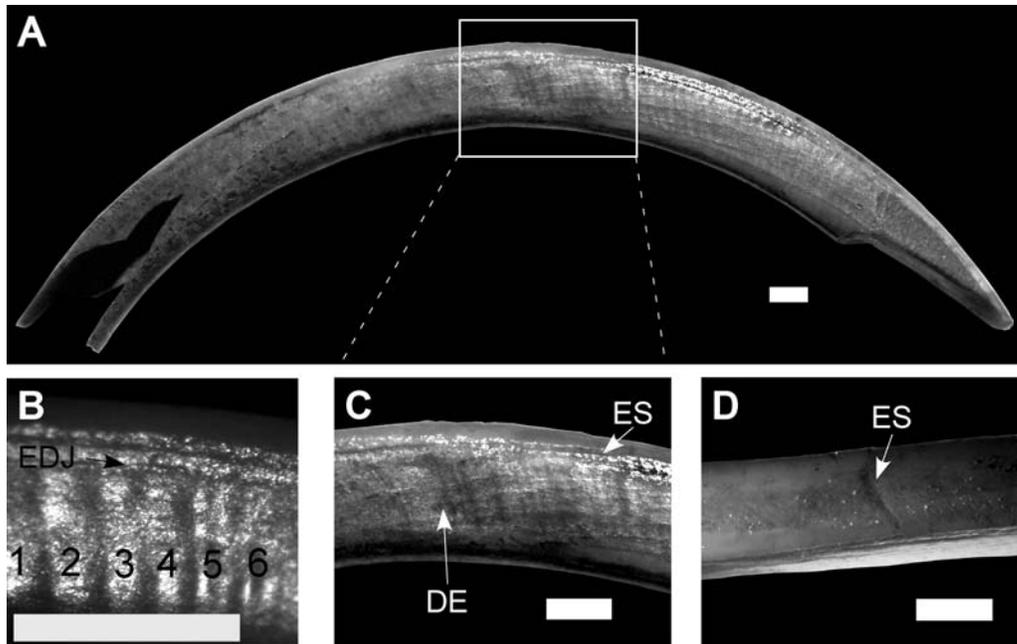


FIG. 1.—Right lower incisors in medial (A, B, and C) and labial (D) views (apex to right in all; labial side up in A–C; medial side down in D). Scale bars are 1 mm. Two specimens are figured: fossil *Spermophilus elegans* (A, C, and D; from Velvet Room, DMNH 12377) and *S. richardsonii* (B; MICH 6537). A) Overview of tooth showing normal incremental dentin and region with hibernation mark (box). B) Magnified view showing 6 dentin increments, with increments 5 and 6 exhibiting a pair of ultradian increments, and doubled reflective bands in enamel labial to the enamel–dentin junction (EDJ). C) Magnified view of region with hibernation mark within box in A, showing diagonal enamel sleeve (ES) and depressed dentine (DE) with fine, poorly developed increments. D) Labial view of enamel sleeve showing convex apical margin.

primarily *S. elegans* (Wyoming ground squirrel) and *S. richardsonii* (Richardson's ground squirrel) with supportive data from *S. parryii* (Arctic ground squirrel). All 3 species exhibit a pronounced annual cycle that includes an obligate, extended period of hibernation lasting 4–8 months depending on age, sex, and species (Buck and Barnes 1999; Fagerstone 1988; Michener 1998). A distinctive surface pattern of dentin and enamel on lower incisors of Pleistocene *S. elegans* was tentatively interpreted as a hibernation mark (Goodwin and Gonzalez 2001), but lack of clear confirmation of this interpretation gave rise to the present investigation. We document the occurrence and morphology of this putative hibernation mark in relation to normal morphology in Recent and fossil samples, confirm its association with hibernation, and explore the utility of event-anchored incremental morphology, combined with tooth-wear data, for inferring life-history and taphonomic events from teeth.

MATERIALS AND METHODS

Specimens and localities.—We examined lower incisors of 231 specimens, including 98 Recent *S. elegans* from Montana (*S. e. aureus*) and Colorado (*S. e. elegans*), 105 Pleistocene *S. elegans* from Porcupine Cave in central Colorado, 3 specimens of *S. parryii* from the Yukon Territory, and 25 specimens of *S. richardsonii* from southern Alberta (Appendix I). The specimens of *S. richardsonii* were individuals with detailed life-history information on age, sex, dates of entry into and emergence from hibernation, and reproductive status that had died of natural causes (usually predation) or accidentally (roadkill) during a long-term behavioral–ecological study (Michener 1992, 2002). Field work with Richardson's ground squirrels followed the guidelines of the

Canadian Council on Animal Care (2003) and the American Society of Mammalogists (Animal Care and Use Committee 1998).

Fossils were from 4 localities within a single chamber of Porcupine Cave: Mark's Sink ($n = 23$), Velvet Room (Denver Museum of Natural History, $n = 59$; most with adequate provenance come from the upper horizons of this stratified site), Velvet Room (Carnegie Museum of Natural History, $n = 10$), and Will's Hole ($n = 13$). Most fossils of small vertebrates were probably brought into this chamber after death by woodrats (*Neotoma*), and in some cases perhaps they were brought in within carnivore scat or raptor regurgitation pellets. The Velvet Room (Carnegie Museum) site was interpreted as a fossilized woodrat midden (Barnosky et al. 2004). Within the temporal framework for Porcupine Cave assemblages (Bell and Barnosky 2000; Goodwin 2004), Mark's Sink is probably mixed in age but includes an old component (early to middle Pleistocene), Will's Hole is probably younger but still middle Pleistocene in age, and the upper levels of the Velvet Room (Denver Museum) and Velvet Room (Carnegie Museum) localities are clearly younger still and probably coeval (Barnosky et al. 2004). The 2 Velvet Room sites were combined in analyses.

Data collection.—Each specimen was assigned to an age class based on eruption of lower cheek teeth; it was deemed to come from an adult if all lower cheek teeth were fully erupted and from a juvenile if not. Degree of wear of cheek teeth was used as an indirect proxy for age, allowing us to categorize specimens into 5 wear categories (1–5, from unworn to heavy wear). For fossil and Recent *S. elegans*, an incisor was extracted from 1 dentary of each lower jaw for full inspection only if already loose; for Recent *S. elegans* with incisors firmly in the jaw, only the visible portion was inspected. For *S. richardsonii* and *S. parryii*, 1 dentary of each lower jaw was soaked in water until the incisor came loose and could be removed for inspection.

All extracted incisors were examined under a stereomicroscope with low-angle incandescent light to visualize enamel and surface in-

crement patterns in dentin. Most specimens were examined with natural contrast, but a few fossil specimens were stained with silver nitrate to enhance increment contrast (following Rinaldi 1999). These observations were used to characterize normal pattern of dentin increments and surface features of enamel and the enamel–dentin junction, the latter a prominent feature at the contact between enamel, which is restricted to the labial surface of the incisor, and transversely oriented dentin increments (Fig. 1B). In addition, features of dentin and enamel previously suggested to be a hibernation mark in fossil *S. elegans* (Goodwin and Gonzalez 2001) were recorded and characterized. The number of days between this mark and the time of death was then inferred, by counting increments from the base of the tooth, which records the last day of life of the individual, to the basal end of the putative hibernation mark. Based on the assumption of daily deposition of enamel and dentin increments (Rinaldi 1999), increment counts on Recent specimens with known dates of death allowed precise estimation of when deposition of the hibernation mark ended. To determine the number of days of growth represented by an entire tooth, counts of dentin increments also were made along the total length of those incisors with adequately visible increments. For specimens with otherwise clear increments, counts within sections of undecipherable increments were quantitatively estimated based on increments per millimeter in adjacent dentin.

Average increment-width values were obtained on 69 specimens, as follows. Specimens were lightly marked medially under magnification by using a china marker pencil in approximately 5-increment (for *S. richardsonii*) or 10-increment segments (for *S. elegans* and *S. parryii*), after which the exact number of increments in each segment was recorded. Specimens were then photographed digitally, with the scale of each digital image calibrated to a ruler included in the photograph. Average increment thickness per segment was calculated as segment length per number of increments from the digital images. The marks were removed by lightly rubbing with a moistened cotton-tipped applicator. Thicknesses of fine, often indistinct, increments in the putative hibernation mark were obtained as above or from scaled camera lucida drawings, which were made for some specimens by using a drawing tube attached to the stereomicroscope.

Distribution of the hibernation mark.—For all Recent samples, we determined the distribution of individuals with the hibernation mark by age (juvenile or adult), sex, and time of year. These distributions allowed us to test our primary working hypothesis that the putative hibernation mark reported by Goodwin and Gonzalez (2001) indeed recorded hibernation and hibernation only. If hibernation is the only cause, then the hibernation mark should never be present in juveniles because they have not yet entered their 1st hibernation; should be present in both sexes, rather than restricted to females as it would be if parturition or lactation were causal; and should occur only in specimens that died during or shortly after hibernation before tooth growth brings the hibernation mark to the apical tip of the tooth where it wears away. The prediction that the hibernation mark is eventually worn away after hibernation was most rigorously tested with *S. richardsonii*, for which exact dates of emergence from hibernation were known.

Life-history and taphonomic analyses.—Correlation between duration of hibernation and length of the hibernation mark, and estimates of average daily dentin deposition rates during hibernation (length of hibernation mark per days of hibernation), were investigated with a sample of *S. richardsonii* ($n = 10$) with known dates of immergence into and emergence from hibernation. Posthibernation dentin growth profiles (average increment thickness versus days after hibernation) were plotted for 13 Recent specimens with ≥ 30 increments of record after hibernation. Profiles were inspected for possible association

between dentin growth rates and posthibernation stressors, such as parturition or onset of lactation.

A model to estimate cohort membership and season at time of death (juvenile, early-season adult, or late-season adult) from jaws with teeth was developed based on samples of Recent *S. elegans* and *S. richardsonii*, by using a combination of cheek-tooth wear and presence or absence of the hibernation mark. This model was then used to explore taphonomic differences across Porcupine Cave fossil sites.

RESULTS

Morphological characterization of incremental dentin and associated enamel.—Dentin increments were evident topographically on the medial surface of all 231 ground-squirrel incisors from fossil and Recent specimens (Fig. 1A), although visualization was difficult on some incisors and impossible in portions of the dentin of other specimens. The topographically elevated portion of an increment sometimes exhibited a pair of yet finer increments (increments 5 and 6 in Fig. 1B) which, when present, often fused near the enamel–dentin junction (increment 6 in Fig. 1B). Incisors with adequately documented increment sequences averaged 64.6 increments per tooth ($SD = 11.9$, $n = 54$), indicating that incisors typically record the previous 65 days of life. The minimum number of increments per tooth in our sample was 42 but most (80% of 54) incisors had 50–80 increments, so we expected that events causing disruption of tooth deposition should be preserved for at least 6 weeks and usually for about 7–11.5 weeks before being lost apically to growth and wear.

Number of increments per tooth did not vary significantly across samples (Table 1), indicating that tooth lifespan was similar in the 3 species and in both fossil and Recent *S. elegans*. However, average increment width per tooth was significantly less for fossil *S. elegans* than for the 2 Recent species with adequate samples (*S. elegans* and *S. richardsonii*; Table 1).

The enamel–dentin junction was sharp, and the enamel just labial to it, in medial view under reflected light, usually exhibited a pair of narrow, adjacent reflective bands coursing parallel to the enamel–dentin junction (Fig. 1B). Some specimens (61 of 231) showed disruption of this structure along a limited segment of the enamel–dentin junction, with a distinct diagonal structure extending from the enamel–dentin junction apically onto the labial surface of the tooth and forming a thickened “sleeve” of enamel clearly delimited from underlying enamel (Fig. 1C). This sleeve of enamel, which we interpreted as a hibernation mark, was present on incisors of fossil (28 of 105) and Recent *S. elegans* (7 of 39 specimens whose incisors we inspected over the full length and 9 of 59 specimens with incisor still in jaw), Recent *S. parryii* (2 of 3), and Recent *S. richardsonii* (14 of 25). The sleeve was usually best visualized medially (Figs. 1A and 1C) but also could often be observed laterally and labially (Fig. 1D). In labial view, the apical margin of the sleeve extended the furthest apically along its medial edge, followed a concave course across the face of the incisor, and typically ended laterally with a minor apical extension (Fig. 1D). Total length of the sleeve from the associated region of abnormal dentin to the sleeve’s apex (see below) was variable ($\bar{X} = 2.57$ mm, $SD = 1.13$, range = 1.10–7.58, $n = 43$); it extended less far

TABLE 1.—Dentin increments and features of the hibernation mark in fossil and Recent *Spermophilus elegans* and in Recent specimens of *S. richardsonii* and *S. parryii*.

	Fossil <i>S. elegans</i>			Recent <i>S. elegans</i>			Recent <i>S. richardsonii</i>			Recent <i>S. parryii</i> ^a			Test statistic ^b	<i>P</i>
	\bar{X}	(SD)	<i>n</i>	\bar{X}	(SD)	<i>n</i>	\bar{X}	(SD)	<i>n</i>	\bar{X}	(SD)	<i>n</i>		
Total increment count per tooth ^c	66.9	(14.5)	18	57.4	(7.5)	10	65.6	(11.1)	23	68.0	(2.7)	3	2.26	ns ^d
Average increment width (mm) ^c	0.28	(0.05)	28	0.39	(0.07)	13	0.37	(0.06)	25	0.40	(0.04)	3	19.24	0.001
Total length of hibernation mark dentin (mm)	1.55	(0.67)	25	1.51	(0.34)	4 ^a	1.46	(0.56)	18	2.20	(0.70)	2	0.46	ns
Length of sleeve from dentin of hibernation mark to apex of sleeve (mm)	2.48	(0.56)	25	1.49	(0.38)	3 ^a	2.97	(1.81)	13	2.68	(0.33)	2	0.95	ns

^a Not included in statistical analysis because of small sample size.

^b Increment count and width compared by analysis of variance, lengths of hibernation mark dentin, and of sleeve compared by Student's *t*-test.

^c Excluding increments in the hibernation mark.

^d ns = not significant.

apically in Recent *S. elegans* than in other samples, but this was not tested statistically because of small sample size (Table 1).

On fully inspected incisors with the sleeve (*n* = 51), the medial surface of the tooth adjacent to the basal end of the sleeve usually exhibited a distinct depression or surface irregularities (*n* = 48) in a zone of homogenous or finely incremented dentin (*n* = 42; Figs. 1A and 1C). When visible, measured increments in this zone averaged much narrower (\bar{X} = 0.15 mm) on most (19 of 20) specimens than increments outside the zone on the same specimens (\bar{X} = 0.32; paired *t* = 8.76, *d.f.* = 19, *P* < 0.001). Length of the zone of abnormal dentin was highly variable (\bar{X} = 1.54 mm, *SD* = 0.61, range = 0.56–3.03, *n* = 49) but did not vary significantly across samples (Table 1).

In addition to the sleeve, 33 of 51 specimens showed enamel abnormalities basal to the sleeve and labial to the zone of abnormal dentin; the abnormal enamel was sometimes thickened, lacked the distinct 2-band pattern described above, and often was deflected downward at the enamel–dentin junction. A few fully inspected incisors (7 of 172) that did not exhibit the enamel sleeve but that did show other correlated features of dentin and enamel were likewise interpreted as exhibiting the putative hibernation mark; the sleeve may have been lost to wear in 1 of these specimens because the hibernation mark was very apically located and lost to chemical erosion in 1 specimen with heavily eroded enamel. Thus, a complex of features consisting primarily of the enamel sleeve but usually with associated anomalies of dentin and enamel is the putative hibernation mark.

Distribution of specimens with the hibernation mark.—In Recent specimens with fully inspected incisors, the putative hibernation mark was never observed in juveniles (*n* = 14) but was commonly present in adult males (13 of 22) and females (15 of 29) of all 3 species. Presence in males rules out a primary role for pregnancy or lactation but is consistent with hibernation as its cause.

Increment counts from the incisor base to the basal end of the putative hibernation mark, anchored to known dates of death on Recent specimens, indicated resumption of normal growth during late winter and early spring (Table 2), as expected if this mark formed during hibernation. In *S. richardsonii*, males resumed normal growth significantly earlier than did females, although this did not occur in a small sample of *S. elegans*. In *S. parryii*, the single male resumed normal growth ~3.5 weeks earlier than the conspecific female (Table 2). Individuals of all 3 species that lived sufficiently late in the active season (>61 days among *S. richardsonii* with known dates of emergence from hibernation) to lose early-season increments to dental growth and wear never exhibited the hibernation mark.

Increment counts (from tooth base to hibernation mark) corresponded well (within ±4 days) with known number of days between emergence from hibernation and death for all female *S. richardsonii* with the hibernation mark (*n* = 10; Fig. 2A). Increment counts were greater than number of days postemergence for 6 of 7 males with the hibernation mark, in 2 cases markedly so (Fig. 2A), likely because final arousal from torpor occurred several days before emergence from the hibernaculum (Michener 1992, 1993). For 3 of 4 radiocollared

TABLE 2.—Estimated dates for end of hibernation for males and females of 3 species of *Spermophilus* shown as mean and range. Dates are based on number of increments from incisor base (most recent dentin deposition) to hibernation mark, assuming that increments were laid down 1 per day.

Taxon	Male			Female			Test statistic ^a	<i>P</i>
	\bar{X}	Minimum–maximum	<i>n</i>	\bar{X}	Minimum–maximum	<i>n</i>		
<i>S. elegans</i>	25 March	8 March–9 April	4	31 March	22 March–5 April	5	–0.94	ns ^b
<i>S. richardsonii</i>	18 February	6 February–1 March	7	19 March	1 March–6 April	10	–4.74	0.001
<i>S. parryii</i>	31 March		1	24 April		1		

^a Student's *t*-test.

^b ns = not significant.

males, increment counts underestimated the known day of final arousal by 4–6 days, suggesting that tooth growth does not resume a circadian pattern while animals are still sequestered in the total darkness of the hibernaculum (Fig. 2B).

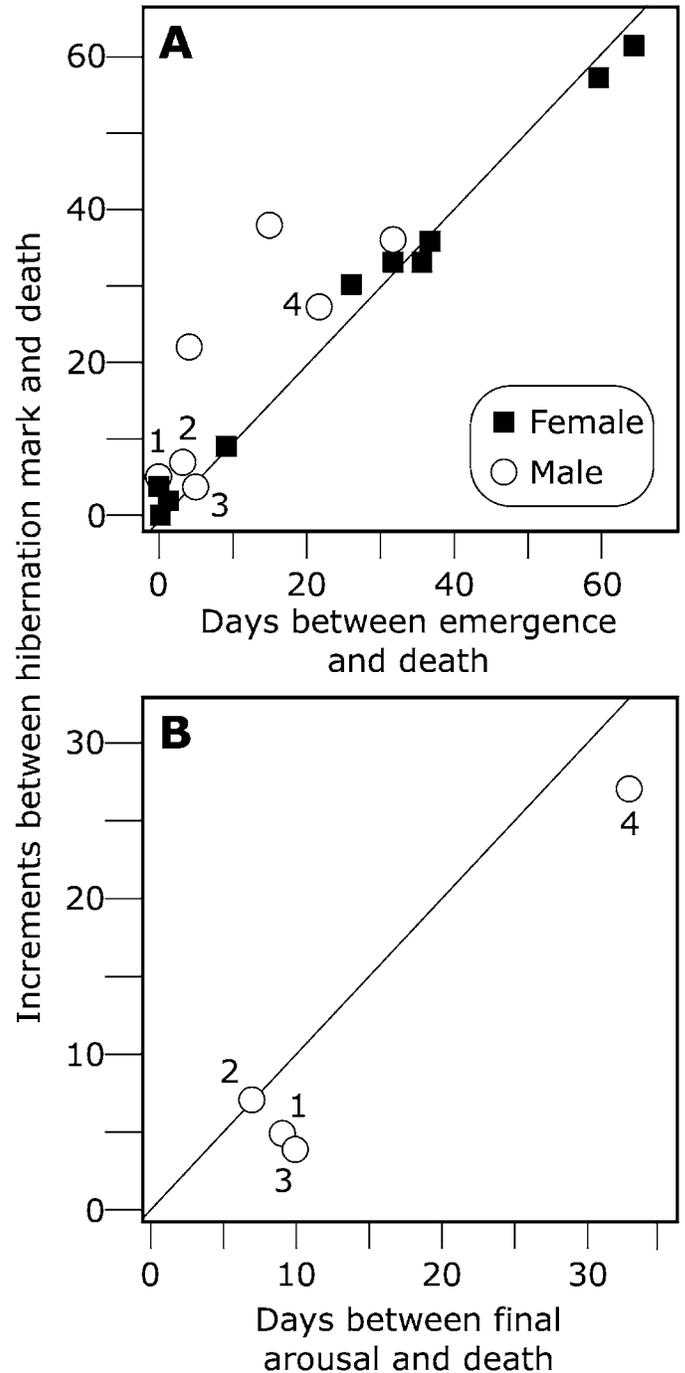
One male *S. richardsonii* killed by a badger 132 days after entry into hibernation (MICH 3208; immersed 11 June 1992, died 21 October 1992) showed a thin but distinct sleeve at the basal end of the incisor, indicating that sleeve development commenced early in hibernation. This specimen also exhibited abnormalities similar to a hibernation mark ~30 increments earlier than this sleeve, including a sleeve-like diagonal discontinuity in medially expressed enamel. Although this was not a classic hibernation mark, it was the only case we observed of a hibernation mark-like morphology forming during the active season.

Dentin deposition during hibernation.—The average rate of dentin deposition during hibernation in *S. richardsonii* (<0.01 mm/day) was <3% of deposition rate during the active season for the same specimens (0.35 mm/day; $n = 10$). Visible increments in the hibernation mark of 2 specimens (counts of 6 and 10) were ~4% of the number of days each spent in hibernation (145 and 264 days, respectively). Length of hibernation-mark dentin showed no association with duration of hibernation ($r = 0.023$, $n = 10$), which ranged from 113 to 281 days.

Posthibernation dentin growth rates in relation to reproduction.—Disruption in tooth growth comparable to that associated with hibernation did not occur during the reproductive season, but dentin growth rates were reduced around the time of parturition (and onset of lactation) in 4 of 5 female *S. richardsonii* with relevant records (Fig. 3A, top 4 panels). This transition occurred ~20–30 increments after hibernation. A similar pattern was evident in some specimens of *S. elegans* (Fig. 3B, top panel). One female *S. richardsonii* showed a spike in growth associated with parturition (Fig. 3A, bottom panel) but died too soon thereafter (4 days) to show sustained response to onset of lactation. One female *S. elegans* showed gradual increase in growth rates across the relevant interval and beyond (Fig. 3C), but we do not know if this individual lactated.

Two male *S. richardsonii* with relevant records showed reduction in growth rates ~15–20 increments after hibernation, with a return to previous growth rates ~10 days later (Fig. 3B, bottom panel), a pattern that coincides with the loss of body mass associated with the stress of the mating season for males (Michener 1984a, 1998; Michener and McLean 1996). One male *S. elegans* (Fig. 3D) exhibits a growth profile closely resembling that of females.

Inferring cohort membership and season at time of death.—The hibernation-mark and age-tooth-wear categories showed a characteristic temporal distribution in Recent *S. elegans* (Figs. 4A and 4B). *S. elegans* with juvenile ($n = 29$) or unworn adult teeth (stage 1; $n = 15$) were collected in June–August and never exhibited the hibernation mark, clearly representing young of year. A single specimen of *S. richardsonii* showed unworn teeth immediately after emerging from hibernation, but with a basally positioned hibernation mark. All specimens with



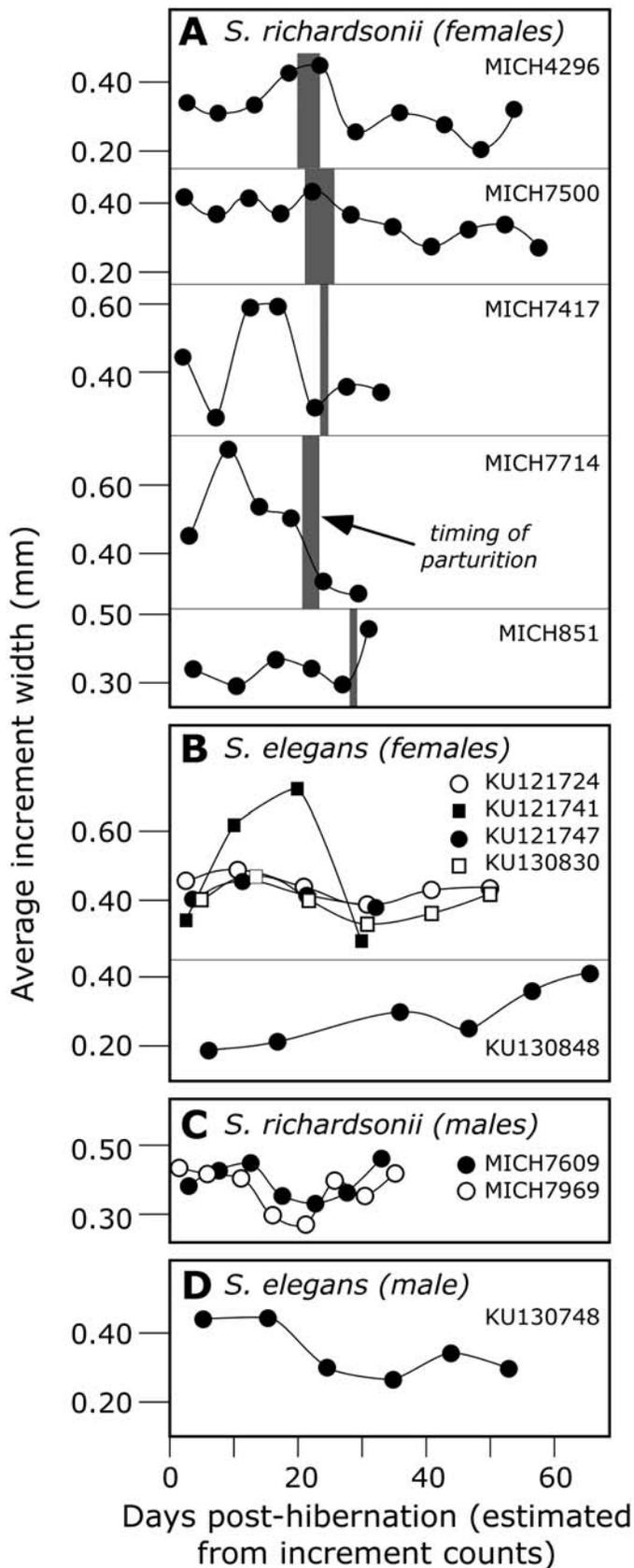


FIG. 3.—Average increment width (per measured tooth segment) plotted against days after hibernation mark (midpoint of each segment; number of increments used as proxy for days) for 13 specimens with

compromised by substantial variation in rates of wear evidenced in a sample of *S. richardsonii* of precisely known age, particularly among yearlings, with males exhibiting greater rates of wear than females (Fig. 5). Individuals with substantial tooth wear (\geq stage 3) but lacking the hibernation mark almost certainly represented adults that died late in the active season after at least 1 hibernation, whereas stage 2 wear (light wear) without the hibernation mark may indicate either late-season young of year or yearling adults active for sufficient days after hibernation for the hibernation mark to have grown out (Fig. 5).

Paleontological utility: estimating cohort and time of death for fossils.—Porcupine Cave samples were graphically distinct from each other in frequency of young of year (juveniles or unworn teeth without hibernation mark), hibernating or early-season adults (hibernation mark preserved), and late-season adults (wear \geq stage 3, without hibernation mark; Fig. 6A). The Will's Hole sample was strikingly divergent (Fig. 6A) in having only young of the year or individuals of uncertain age that could be young of year (wear stage 2 with no hibernation mark). Velvet Room (combined sample) and Mark's Sink samples showed greater similarity to each other in proportions of cohorts and seasons of death (Fig. 6A), but differed markedly in time of death relative to arousal from hibernation for specimens with the hibernation mark (Fig. 6B). Peak frequency of Velvet Room deaths was during or immediately after hibernation (0–9 days after hibernation), whereas the peak for Mark's Sink was much later (50–59 days after hibernation). Seven of 9 individuals in the 0- to 9-day category showed no clear posthibernation increments, implying death during or immediately upon arousal from hibernation. Three of these individuals (specimens DMNH 12143, DMNH 6614, and CMNH 49251) exhibited a thin, basally positioned sleeve very similar to that seen in a specimen of *S. richardsonii* that died during hibernation (MICH 3208).

DISCUSSION

Incisor dentin is normally laid down in a regular daily pattern in rodents (e.g., Klevezal 1996; Rinaldi 1999; Schour and Steadman 1935). Although metabolic stressors such as lactation may depress rates of dentin deposition (Klevezal 1996), examination of our data indicates that the circadian periodicity of deposition is maintained in *S. richardsonii* through parturition and lactation (Fig 2A). In contrast, because

←

\geq 30 increments after the hibernation mark. A) Female *Spermophilus richardsonii* with posthibernation increments extending past known date of parturition (parturition displayed as gray bar; thickness records uncertainty of placement due to slight mismatch between increment counts and known days since emergence; see Fig. 2A). B) Female *S. elegans* displaying reduced increment width \sim 20–25 days after hibernation (top panel) or not (bottom panel). C) Male *S. richardsonii*. D) Male *S. elegans*. (Specimen numbers on graphs are for specimens from collection of G. Michener [MICH] or University of Kansas Museum of Natural History [KU]—Appendix I.)

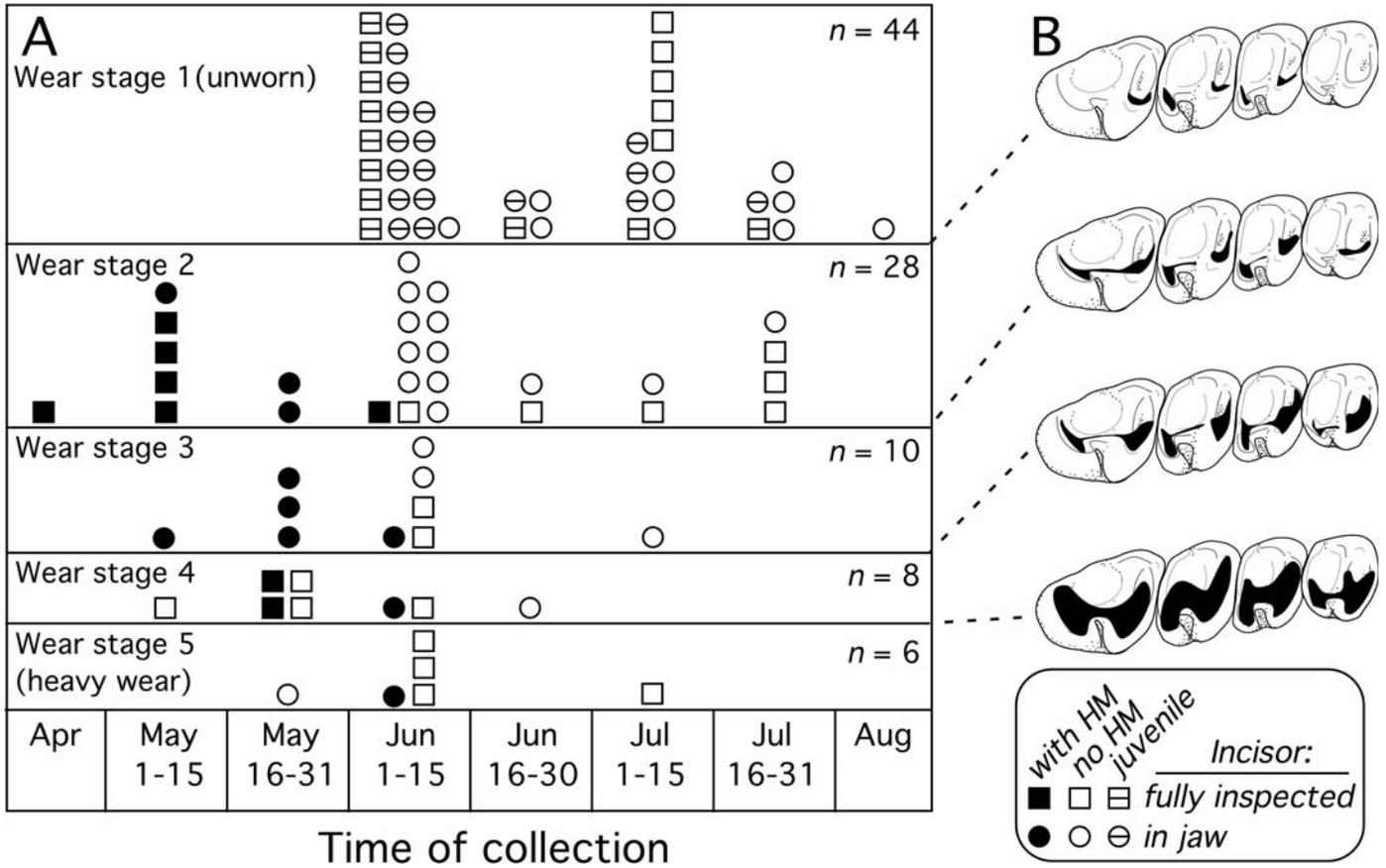


FIG. 4.—A) Distribution of 96 specimens of *Spermophilus elegans* with and without hibernation mark (HM) in incisors relative to time of collection and cheek-tooth wear. B) Graphic depiction of tooth wear at boundaries between wear categories.

hibernation lasts many months and is marked by significant reduction of physiological activity, metabolic depression during hibernation should produce a conspicuous interruption in dentin deposition. Adult *S. elegans* hibernate for about 8–9 months and juveniles for 7.5 months (Fagerstone 1988). Adult *S. richardsonii* likewise hibernate for 8–9 months, juvenile females for 6–7 months, and juvenile males for 4–5 months (Michener 1998, 2002). Male and female *S. richardsonii* spend about 85% and 91%, respectively, of hibernation in the physiological state of torpor (Michener 1992), during which time metabolic rate is drastically reduced (Wang 1979). Both adult and juvenile *S. parryii* hibernate for about 7–8 months (Buck and Barnes 1999). Our study confirms that hibernation is reflected in incisor dentin and enamel in these 3 species of *Spermophilus*.

A putative hibernation mark, previously documented in fossil *S. elegans* (Goodwin and Gonzalez 2001), is present in Recent specimens of *S. elegans*, *S. richardsonii*, and *S. parryii* and is exclusively associated with hibernation. Some aspects of the hibernation mark, such as the typically narrow increments (reduced appositional growth rates) and depressed medial dentin sequence (reduced outward growth of the dentin cone) within the hibernation mark, are clearly explicable based on drastic reduction in metabolic rate during torpor (Wang 1979). The origin of the most distinctive feature of the hibernation

mark, the enamel sleeve, is less clear. A tentative interpretation is that enamel deposition is not slowed as much as dentin deposition during hibernation. Because the tooth is not erupting rapidly, but enamel is still being deposited appositionally, a thickened sleeve of enamel forms above prehibernation enamel. The somewhat concave apical front of the sleeve, in labial view, is roughly similar to a broadly C-shaped front of ameloblast activity observed in developing rat incisors (Smith

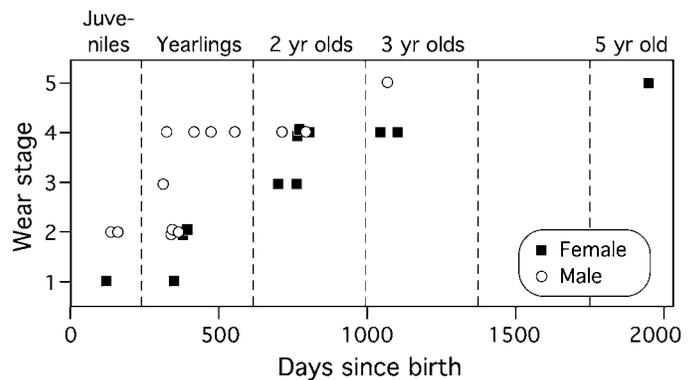


FIG. 5.—Relationship between wear stage of cheek teeth and number of days since birth for known-age male and female *Spermophilus richardsonii*. Symbols are offset vertically when tightly clustered to enhance visibility.

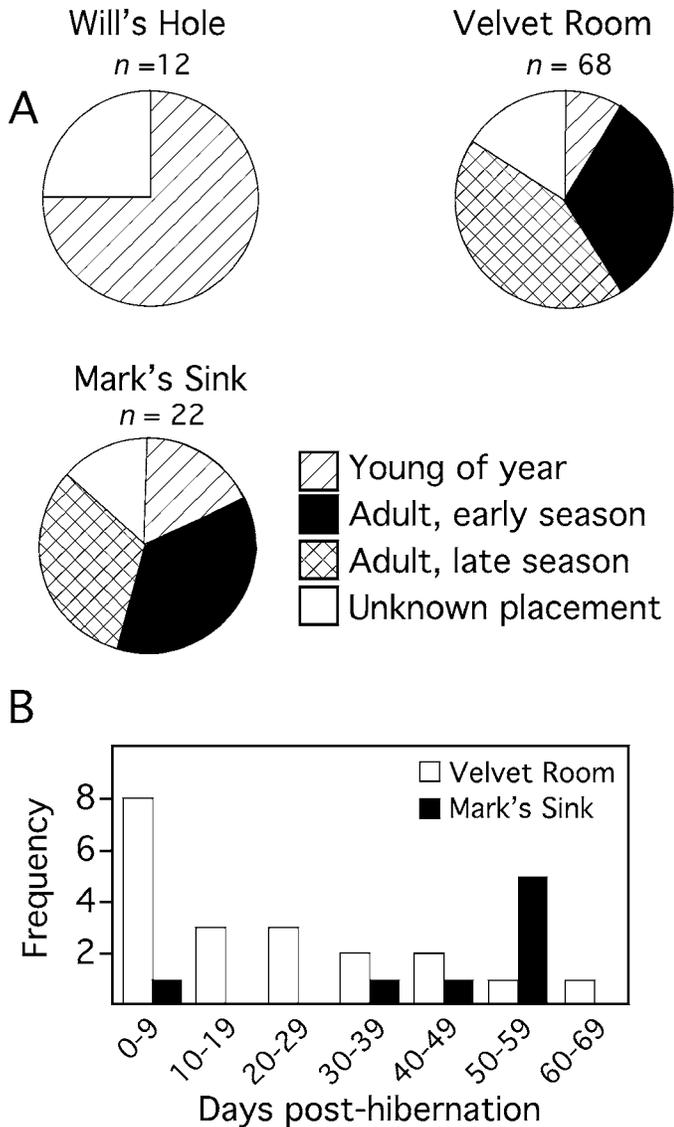


FIG. 6.—A) Relative distribution of specimens of fossil *Spermophilus elegans* in 4 age–season categories in 3 samples within Porcupine Cave (Velvet Room sample combines specimens from Carnegie Museum of Natural History and Denver Museum of Natural History localities). Categories are inferred as follows: juvenile dentition or unworn teeth without hibernation mark = young of year; any specimen with hibernation mark evident = adult taken early in the season (before the hibernation mark could wear out); specimens with wear stage 3 or higher and lacking hibernation mark = late-season adults (died sufficiently late in the season that the hibernation mark was worn away); specimens with wear stage 2 and no hibernation mark = unknown placement (either late-season young of year or late-season adults after their 1st hibernation). B) Temporal distribution of latency between termination of hibernation and death determined from increment counts in incisors for specimens with a hibernation mark from Velvet Room and Mark’s Sink localities.

and Warshawsky 1976), although the lateral margin of the front extended most apically in rats compared with the medial margin for the sleeve in ground squirrels.

The few visible increments within the hibernation mark clearly do not reflect circadian periodicity. It is tempting to

speculate that they reflect the number of bouts of arousal during hibernation, although such a relationship was not evident for *Marmota* (Rinaldi 1999). Close correspondence between post-hibernation increment counts and known days since emergence for female *S. richardsonii* (Fig. 2A) supports resumption of circadian periodicity for normal increments. Posthibernation increment counts typically underestimate days posttorpor for males that terminate torpor but remain in the hibernaculum for several days before emerging, suggesting that circadian periodicity is not reestablished until animals resume aboveground activity. The presence of “doubled” increments that are almost certainly ultradian in frequency (Fig. 1B) indicates that care must be taken in counting increments. Difficulty in distinguishing ultradian and circadian increments in fossil *S. elegans* may explain the significantly narrower increments observed in that sample (Table 1) and thus may compromise estimates of days of record per tooth in that sample.

Presence of the hibernation mark in 3 species of *Spermophilus* and 1 species of *Marmota* in the tribe Marmotini implies a broad distribution. Further work is needed on rodent hibernators within and outside Marmotini to document the full range of dental expression of hibernation and to understand the underlying factors that determine deposition of dentin and enamel.

The ability to detect important life-history events after the fact by surface inspection of teeth has clear utility, albeit with limits. For example, increment-width profiles may record reduced dentin growth with the stress of mating in males and onset of lactation in some female *S. richardsonii*. Hibernation involves significant metabolic depression over long periods of time, and this may be what is required to produce a diagnostic surface signal. Possible applications for paleontologists and archaeologists include direct investigation of the fossil record of hibernation and taphonomic questions that require relatively precise evidence of season of death. The potential to micro-sample incremental series for isotopic analysis may facilitate fine-scale paleoenvironmental interpretations. Additionally, wildlife biologists might use the association between hibernation and dental morphology to infer approximate time of final arousal before emergence in field studies. Male *S. richardsonii* frequently terminate torpor several days before they emerge from hibernation, particularly if they have access to a cache of seeds within the hibernaculum chamber (Charge 2001; Michener 1993), and males of other species likely do so as well. Tooth morphology might provide a substitute to the use of radiotelemetry to infer the occurrence of a latency between termination of torpor and emergence from hibernation, although increment counts in this study typically underestimated the magnitude of this latency by 4–6 days (Fig. 2B).

Investigations at Porcupine Cave exemplify taphonomic interpretations dependent in part on ability to detect a hibernation mark. The presence of a very thin, basally positioned sleeve in some specimens and the lack of clear posthibernation increments in other specimens of *S. elegans* from the Velvet Room sites suggest death during or shortly after hibernation. Because many fossils in Porcupine Cave were probably carried in by woodrats (Barnosky et al. 2004), it is puzzling how the

jaws of hibernating *S. elegans* became available for woodrats to collect. The presence of mostly young of the year (based on wear and absence of the hibernation mark) in the Will's Hole sample of *S. elegans* (Fig. 5A) suggests that this collection was made during a limited time interval, late in the active season when young were still active but most adults had immersed, a pattern of activity characteristic of many hibernating species of *Spermophilus* (Michener 1984b). Similar taphonomic questions may be addressed for other Pleistocene and perhaps older fossil sites given the ability to assess time of death relative to hibernation from surface morphology of *Spermophilus* incisors.

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APPENDIX I

Specimens examined for this study. Specimens with a hibernation mark are marked with an asterisk (*); those with only distal tip of incisor visible (remainder of incisor in jaw) are marked with a dagger (†). Specimens are from the following collections (noted by corresponding acronyms): Carnegie Museum of Natural History,

Pittsburgh, Pennsylvania (CMNH); Denver Museum of Natural History, Denver, Colorado (DMNH); University of Kansas Museum of Natural History, Lawrence, Kansas (KU); University of Michigan Museum of Zoology, Ann Arbor, Michigan (UMMZ); and the personal collection of Gail R. Michener (MICH).

Spermophilus elegans: *Recent*.—KU collection. *S. e. elegans*, all from Colorado: 8608†, 8609†, 8610†, 19993, 19994†, 19995†, 59094†, 59765†, 116908†, 126785†, 126786†, 126787†, 130850†, 130851†, 130852†, 130853†, 130854†, 130855†, 130856†, 130857†, 130858*†, 130859*†, 130860†, 130861†, 130862, 130863†, 130865†, 149689†, 149690, 149691.

S. e. aureus, all from Montana: 121719*†, 121720*†, 121721†, 121722, 121723, 121724*, 121725, 121726, 121727, 121731†, 121732*†, 121735, 121737, 121738, 121740, 121741*, 121743*, 121747*, 128292, 130748*, 130750*†, 130752*†, 130763*†, 130766, 130769, 130783, 130795, 130801, 130826*†, 130829*†, 130830*, 130831, 130835, 130836†, 130838†, 130839†, 130840†, 130842†, 130843†, 130844, 130845†, 130846†, 130847†, 130848*, 130864†, 130872†, 130873†, 130874, 130875, 130876, 130877†, 130879*, 130880*, 130881†, 130882†, 130883, 130884, 130885, 130886†, 130887†, 130888†, 130889†, 130890†, 130891†, 130892†, 130893, 130894, 130895†.

Spermophilus elegans: *fossil*.—All from localities within Porcupine Cave, Park County, Colorado.

Mark's Sink (DMNH collection): 22469*, 22471, 22513, 22543, 22545*, 22550, 22551, 22556, 22575*, 22577, 22593, 27531*, 27535, 33517, 33518*, 33519*, 33535, 33848, 36922*, 37109*, 37116, 37208, 37213.

Velvet Room—Denver Museum of Natural History site (DMNH collection): 6489, 6508, 6596, 6614*, 6616, 6685, 6727, 8206*, 8318*, 8322, 8420, 8543, 8548, 8549, 9646, 9650, 9653, 9716*, 9861*, 9875, 9937, 9945, 9968, 10516*, 10526, 10612*, 10746, 10909, 11077, 11171, 11173, 11211*, 11256, 11820, 11934, 12143*, 12151, 12209*, 12218, 12241, 12298*, 12337*, 12377*, 12540, 12544*, 12551, 12555, 12556*, 20851, 20852*, 20985*, 21559, 21560*, 21561, 21605, 21853, 22404, 23215, 23217.

Velvet Room—Carnegie Museum of Natural History site (CMNH collection): 49202, 49207, 49213, 49227, 49244*, 49250, 49251*, 49266, 49268*, 49272*.

Will's Hole (DMNH collection): 22869, 22883, 22884, 22885, 22887, 37256, 37269, 37282, 37289, 37290, 37291, 37295, 37297.

Spermophilus parryii.—UMMZ collection. All from Yukon Territory: 158169*, 158170*, 158181.

Spermophilus richardsonii.—MICH, deposited at KU but not yet accessioned. All from southern Alberta: 850, 851*, 878*, 954*, 1544*, 1673*, 2333, 3208, 3725*, 3804*, 4296*, 5688, 6051*, 6353*, 6537, 6620, 6651*, 6841, 6855*, 7417*, 7500*, 7609*, 7714*, 7969*, 8464.