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Age Determination and Age-Specific Reproduction in Belding's Ground Squirrels

Abstract

Numbers of densely stained lines in mandibles of more than 95 percent of marked Belding's ground squirrels (*Spermophilus beldingi*) corresponded with their known ages or known minimum ages; seemingly, one new line was formed each year, and evidence of resorption of lines was absent. Mean litter sizes, based on counts of embryos or pigmented sites of implantation, for 273 females collected in 1975 and 1976 on the Oregon State University Harris Ranch, Grant County, Oregon, and 311 females obtained from hunters during the same year on the adjacent Izee Ranch, increased with age in five of six samples. Mean litter sizes were significantly larger among 2-year-old squirrels than among 1-year-olds in five of six samples but were not significantly larger than among 3-year-olds in three of five samples. Because few squirrels had three densely stained lines and none had four or more, and because differences in mean litter sizes between second-cohort and older breeders were frequently not significant, estimates of natality for squirrels on our study areas, based only on yearling and older age categories (common in earlier studies), would not be greatly in error.

Introduction

Although natality in Belding's ground squirrels was estimated by several investigators (Grinnel and Dixon 1919, Bailey 1936, Howell 1938, McKeever 1964, Turner 1972, Sauer 1976), none classified ages of maternal females more precisely than to yearling and older categories. However, a relationship between maternal age and litter size was reported among several sciurid rodents (Slade and Balph 1974, Nixon and McClain 1975) and other mammals (Nalbandov 1976). Morton and Gallup (1975) reported that female *S. beldingi* 2 or more years old produced significantly more offspring than yearling females, but they did not separate the older squirrels into year classes. Because Sherman (1977) reported wild adult *S. beldingi* females in 2- to 8-year age classes, we believed that investigation of age-specific natality in a population of the species was warranted.

In earlier studies, division of the adult cohort into year classes seemingly was possible only by repeatedly livetrapping, over a period of years, individuals marked as juveniles (Morton and Parmer 1975, Morton and Gallup 1975). Klevezal' and Kleinenberg (1967) reported that lines in periosteal bone that stained more darkly than surrounding bone was formed annually in numerous, but not all, species of mammals; they referred to these lines as "adhesion lines." Millar and Zwickel (1972), Sullins *et al.* (1976), and Frylestam and von Schantz (1977) found adhesion lines useful in estimating ages of several lagomorphs; Livezey and Verts (1979) found a relationship between numbers of adhesion lines and ages determined by other criteria in geo-

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myids. To our knowledge, determination of age by counts of densely stained lines in bones of sciurid rodents in the western hemisphere was not attempted previously. If applicable, the technique would permit more precise estimates of natality in *S. beldingi*, a species of considerable economic importance (Sullins and Verts 1978).

Methods

During April-June 1975 and April-May 1976, 105 and 168 female Belding's ground squirrels were collected on the Oregon State University Harris Ranch (elevation = 1700 m) approximately 63 km southwest of John Day, Grant County, Oregon, respectively. In addition, 158 and 153 female squirrels were obtained from hunters who participated in an annual ground squirrel shoot on the adjacent Izee Ranch on 30 May 1975 and 1976, respectively.

The uterus of each squirrel was excised and examined for developing embryos and pigmented sites of implantation; viable and resorbing embryos were recorded separately. Mandibles were removed, defleshed, and air dried. In the laboratory, mandibles were decalcified in 5 percent nitric acid for 12 hours, or until flexible, and rinsed for 12 hours in running water. A segment from each mandible anterior to the molariform row was removed and cross-sectioned at 20-25 μ on a freezing microtome. Several sections from each mandible were mounted on slides with Mayer's albumen, stained with Papanicolaou (Harris) hematoxylin, and examined under a microscope for darkly stained lines. Numbers of darkly stained lines were recorded.

To determine if numbers of darkly stained lines were related to age, mandibles from 49 Belding's ground squirrels livetrapped, classified as young-of-the-year or older, marked, and released on the Harris Ranch in 1975 were collected in 1976. In addition, mandibles from 23 squirrels judged young-of-the-year on the basis of size and weight (Turner 1972) were collected in 1976. Mandibles were prepared and examined as described earlier; we expected those judged young-of-the-year to have no darkly stained lines, those marked as young-of-the-year and examined as yearlings to have one stained line, and those marked as older than young-of-the-year in 1975 to have two or more lines.

Student's *t* test or analysis of variance was used to ascertain the probability that numbers of embryos or implantation sites per female between age classes were significantly different depending on whether two or more than two age cohorts of females occurred in samples. In instances that analysis of variance indicated $P < 0.05$, Fisher's protected least significant difference test was used to ascertain which mean rates were significantly different.

Results and Discussion

Among the 23 young-of-the-year ground squirrels collected in 1976, all possessed mandibles without stained lines; among those marked in 1975 and collected in 1976, 20 (95.2 percent) of 21 yearlings had one line and 27 (96.4 percent) of 28 older squirrels had two or more lines. Apparently, one line was formed each year in *S. beldingi*; no squirrel exhibited evidence of resorbing adhesion lines.

Absence of stained lines in mandibles of 18 adult ground squirrels collected on the Harris Ranch in 1975 and 1976 (Table 1) seemed inconsistent with this conclu-

TABLE 1. Numbers of adult female Belding's ground squirrels with 0, 1, 2, and 3 adhesion lines in their mandibles in each collection, 17 April-20 June 1975 and 7 April-31 May 1976, Harris Ranch, Grant County, Oregon.

Date of collection		Squirrels examined (N)	Number of adhesion lines			
Year	Day and Month		0	1	2	3
1975	17 April	23	8	10	5	
	21 April	21	2	11	8	1
	3 May	19	1	10	7	1
	10 May	16		11	5	
	20 June	25		17	8	
1976	7 April	24	6	6	12	
	14 April	24	1	12	10	1
	26 April	19		14	3	2
	2 May	27		11	14	2
	11 May	28		15	8	5
	18 May	21		10	9	2
	31 May	25		8	15	2

sion. These adults were collected before juveniles emerged from natal dens; hence, they were known to be at least 1 year old. Because similar discrepancies were not noticed among collections of squirrels made later in the season during both years (Table 1), we suggest that deposition of bone tissue sufficient to distinguish darkly stained lines from the stained outer surfaces of the mandibles had not occurred in these squirrels, a supposition consistent with those of Klevezal' and Kleinenberg (1967). Seasonal differences in proportions of female squirrels without discernible stained lines in their mandibles (Table 1) possibly were related to differences in phenology between 1975 and 1976 (Costain 1978). Because 17 (94.4 percent) of 18 squirrels without stained lines were nulliparous whereas only 17 (6.7 percent) of 255 with lines were nulliparous, we suspect that females without lines were collected shortly after they emerged from dormancy and before their first estrus, or possibly some were late-born young of the previous year that did not breed as yearlings.

We believe that the strong relationship between numbers of darkly stained lines in mandibles of ground squirrels and numbers expected on the basis of their known ages or known minimum ages, and evidence that individuals whose darkly stained lines were not discernible were assignable to the 1-year adult cohort, is justification for separating samples of Beldin's ground squirrels into age classes by the periosteal zonation technique.

TABLE 2. Numbers of implantation sites ($\bar{X} \pm S.E.$) for parous¹ Belding's ground squirrels with 0, 1, 2, and 3 adhesion lines in periosteal zones of their mandibles, Izee Ranch, Grant County, Oregon, 30 May 1975 and 1976.

Adhesion lines (N)	1975		1976	
	Females examined (N)	Implantation sites ($\bar{X} \pm S.E.$) ²	Females examined (N)	Implantation sites ($\bar{X} \pm S.E.$) ²
0	0		0	
1	97	6.0 \pm 0.2	82	6.4 \pm 0.2
2	51	6.6 \pm 0.2 ^a	66	7.1 \pm 0.2
3	3	8.0 ^a	3	10.3

¹In 1975, seven females with one adhesion line were nulliparous; in 1976, two with one adhesion line were nulliparous.

²Means within a collection and in the same column followed by the same letter were not significantly different ($P > 0.05$).

TABLE 3. Reproductive performance of female Belding's ground squirrels with 0, 1, 2, and 3 adhesion lines in periosteal zones of their mandibles, Harris Ranch, Grant County, Oregon, 1975-76.

Category	Number of adhesion lines (1975)			Number of adhesion lines (1976)				
	0	1	2	3	0	1	2	3
Adult females examined (N)	11	59	33	2	7	76	71	14
Females nulliparous (N)	11	3	4		6	7	3	
Females pregnant (N)		31	18	2	1	29	26	3
Postpartum females (N)		16	11			40	42	11
Viable embryos/pregnant female (X±S.E.) ¹		5.1±0.2a	5.8±0.4a	6.5	5	4.5±0.2b	5.9±0.2	3.7b
Females with resorbing embryos (N)		4	3			6	5	
Resorbing embryos/pregnant female (X)		0.1	0.2			0.3	0.3	
Implantation sites/postpartum female (X±S.E.) ¹		4.4±0.3	5.5±0.3			5.1±0.2	5.9±0.2c	6.9±0.3c

¹Means within a collection and in the same row followed by the same letter were not significantly different ($P > 0.05$).

Except for third-cohort pregnant females on the Harris Ranch in 1976, mean litter sizes based on counts of viable embryos and implantation sites increased with maternal age (Tables 2 and 3); analysis of variance indicated differences within collections were significant ($P < 0.05$), except those for pregnant females obtained on the Harris Ranch in 1975 (Table 3). Seemingly, differences in mean numbers of embryos or implantation sites between first- and second-cohort breeders were responsible for significant differences within collections; in only two of five instances were mean numbers of embryos or implantation sites significantly different between second- and third-cohort breeders (Tables 2 and 3).

Because numbers of Belding's ground squirrels in cohorts with more than two adhesion lines were low, and because differences in litter sizes between second-cohort and older breeders frequently were not significant, we believe that estimates of natality for squirrels on our study areas based on only two age categories (yearlings and older squirrels) would not be greatly in error.

Although Sherman (1977) did not report the proportion of the 1135 *S. beldingi* that he marked that were in each sex and age class, we suspect that the number of females older than 3 years was relatively small. However, the relative infrequency of ground squirrels with three adhesion lines and the absence of squirrels with four or more lines (Tables 2 and 3) suggested that the dynamics of populations of Belding's ground squirrels on our study areas were somewhat different from those in California that Sherman (1977) studied. We speculate that reduction of numbers of ground squirrels by routine control programs (Sullins and Verts 1978) or the annual ground squirrel shoot truncated age distributions of squirrels on our study areas.

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