

# Abert's Squirrels of the Colorado Plateau: Their Reproductive Cycle

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**Abstract.** Results of a morphological and histological analysis of Abert's squirrels (*Sciurus aberti aberti*) killed on the road are presented. Resolving either the sex or the age of the animals on the basis of body measurements is not possible. Plotting testis weight against body length, however, seems to differentiate between juvenile and adult animals. Testicular activity, as judged from weight increases and histological analyses, displays a broad pattern that begins in October and ends in May–June when testicular regression ensues. Testicular regression is not complete because some residual meiotic activity is retained even through September and October. This maintenance meiosis serves to preserve the testes in a state of preparedness from which rapid recrudescence can be implemented. Evidence for dual gestation in Abert's squirrels is indicated by actual embryos and the presence of lactating nipples.

**Key words:** Embryos, gestation, reproduction, spermatogenesis, testis.

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Ponderosa pine (*Pinus ponderosa*) forests of Arizona provide habitats for Abert's squirrels. These animals make extensive use of the pine trees for shelter, nest material, and food (e.g., inner bark of pine shoots and seeds from ovulate cones; Keith 1965; Patton 1975; Allred 1989). Although inner bark is available year-round, its caloric content varies seasonally from 2,454 cal/g in December to 4,999 cal/g in May (Pedersen et al. 1987) and may determine the use of alternate food sources. Abert's squirrels also act as spore-dispersing agents when feeding on ectomycorrhizal fruiting bodies growing in association with ponderosa pine roots (Kotter and Farentinos 1984; States et al. 1988). Dispersal of trees may be aided by these animals when they bury ovulate cones that are then left untouched (Bailey 1932).

Abert's squirrels are food for goshawks (Reynolds 1963; Reynolds et al. 1992) and therefore serve as important intermediates in the food chain of the

ponderosa pine forest. Although the density of squirrel populations is influenced by food resources (Keith 1965) and climate (Stephenson and Brown 1980), the reproductive mechanisms responsible for maintaining population levels are still elusive. Some authors suggest that these animals have only a single reproduction period (Farentinos 1972, 1980; Brown 1984), whereas others propose that, under certain circumstances, two gestations in one season are possible (Hall and Kelson 1959; J. Hall, 1992, personal communications). We present morphological and histological data that provide a comprehensive view of the reproductive cycle in Abert's squirrels. Tangible evidence for two periods of gestation in one season is also offered.

## Methods

Carcasses were recovered from roads and kept frozen until examined. Most animals were obtained from the immediate Flagstaff vicinity that varied by no more than 61 m in elevation (average 2,164 m) over a 129-km<sup>2</sup> area. Animals were thawed at room temperature overnight and weighed. Measurements included total length, hind foot, tail, ear, and tassel length. Male sexual organs (testes and attached epididymides) were removed from the carcasses, weighed, and fixed in Bouin's fluid for 24 h. Tissues were then processed for histological examination using standard methods of dehydration through alcohols, paraffin infiltration, sectioning, and staining with haematoxylin and eosin. Female tissues were similarly excised and processed. When possible, the ovaries, oviducts, and the upper segment of the uterus were included.

All photographs were obtained with Plus X film through a Zeiss microscope equipped with an automatic camera system (MC 63). The negatives were developed in D76 (1:1) and printed on Ilford RC multigrade paper.

## Results

We present data from 2 years of a continuing collection that began in February 1992. Morphological parameters plotted in a variety of combinations yield only scatter diagrams that cannot be resolved either into the sexes or the ages of the squirrels (Fig. 1). Adult and juvenile squirrels, however, may be distinguishable from each other on the basis of total body weight (Fig. 2). For any one body length, the right testis can either be heavy (probably mature) or

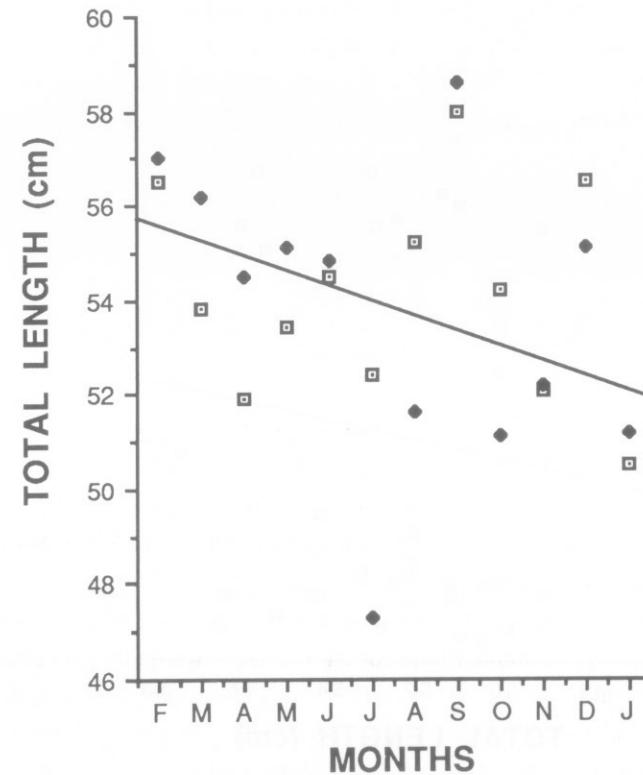


Fig. 1. Total length of squirrels plotted against the month of collection. Months are represented by the first letter beginning with February (F). *Open squares* represent males and *diamonds* represent females. The *line* represents the best-curve fit.

light (possibly from a juvenile). Therefore, body length alone does not provide information on the age or sex of squirrels. A similar plot can be obtained if testis weight is plotted against total body weight (not shown).

If testicular activity is correlated with increases in weight then our data reveal a broad peak of testicular enlargement and activity extending from September–October to June (Fig. 3). Testicular regression occurs during July and August. During that time, meiotic divisions and spermatogenic elements such as primary spermatocytes are seen within the germinal epithelium (Fig. 4). Resumption of spermatogenesis is synchronized with the emergence of second-

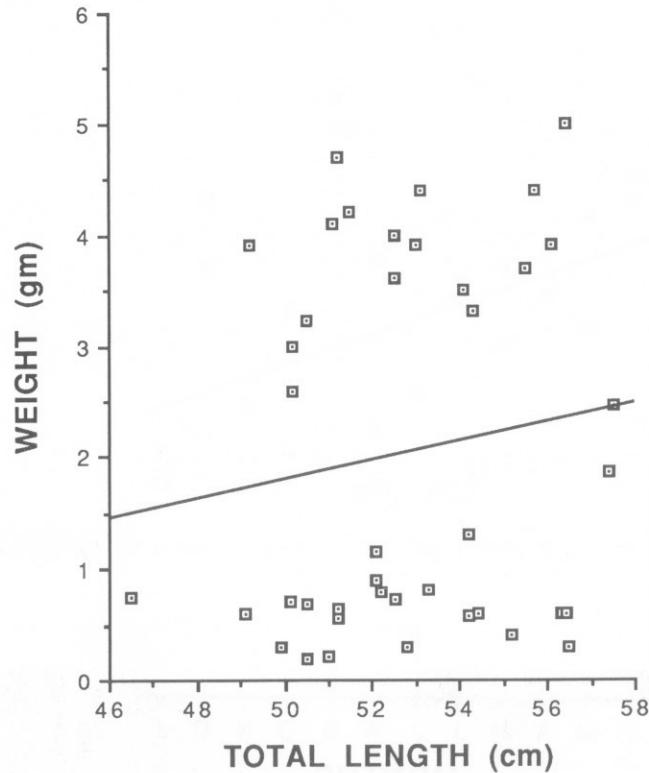


Fig. 2. Distribution of the weight of the right testis plotted against total length. The line represents the best-curve fit.

primary spermatocytes and spermatids. Maximal spermatogenic activity occurs through March and April (intermediate months not shown) and is indicated by the production of mature sperm, their release into the lumen of seminiferous tubules (Fig. 5a), and their accumulation within the vas deferens (Fig. 5b). The presence of large numbers of sperm within the vas deferens in June (Fig. 6) is interpreted as the latest time in the season when males can impregnate.

Testicular regression occurs abruptly and is already manifest in July. At that time, collapsed seminiferous tubules, devoid of sperm, are visible (Fig. 7). Both the thickness and the cellular complexity of the germinal epithelium in

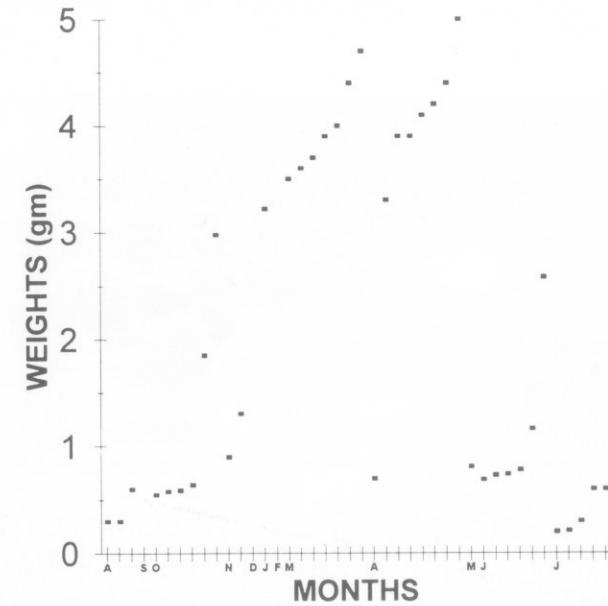


Fig. 3. Right testicular weights plotted against the month of collection. The months are represented by the first letter beginning with August (A).

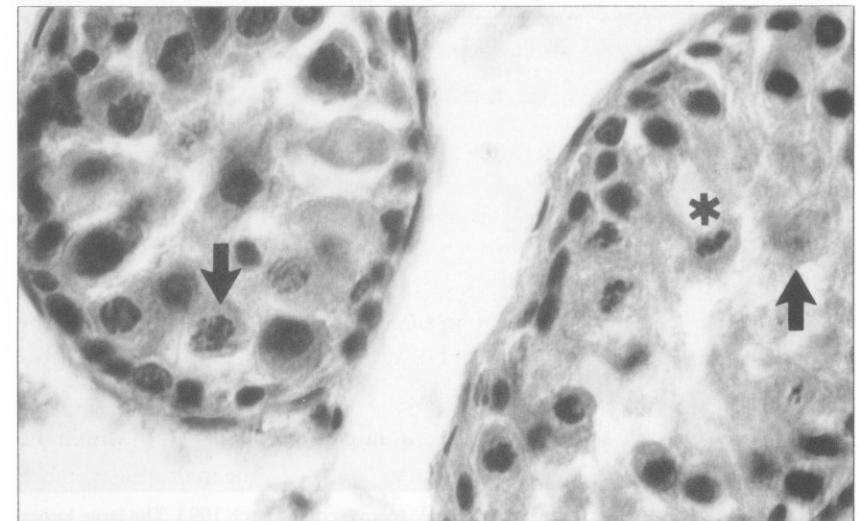
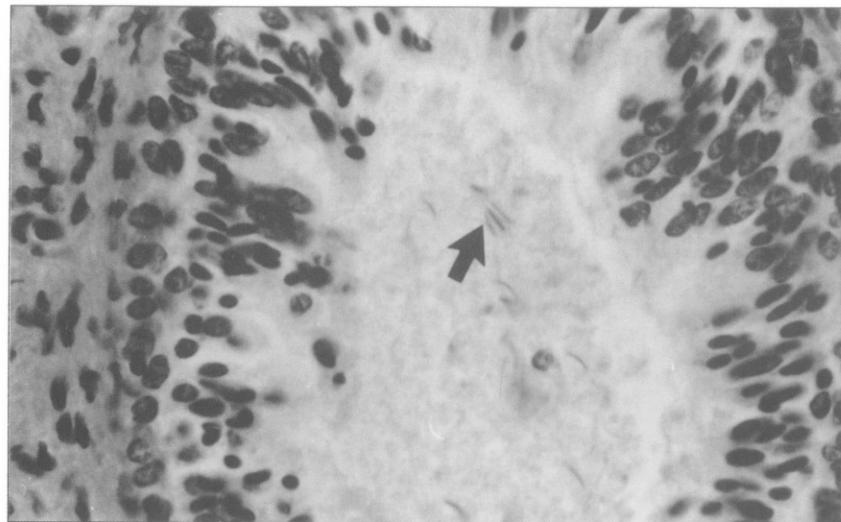
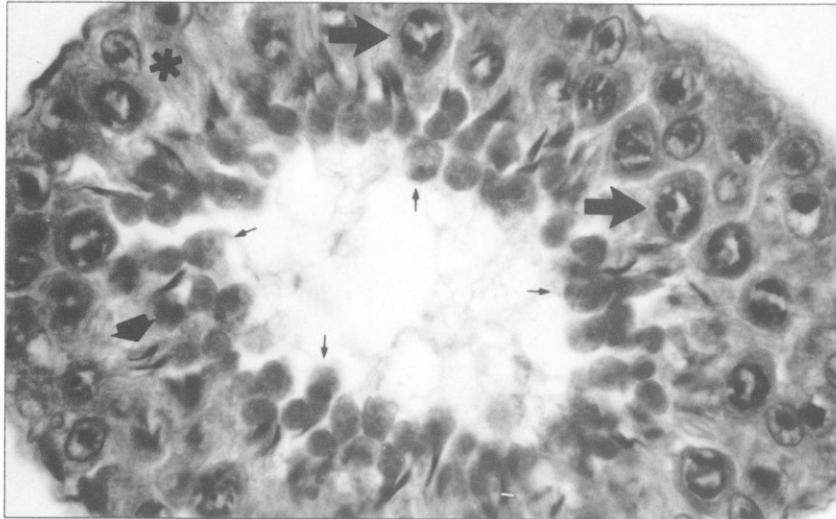
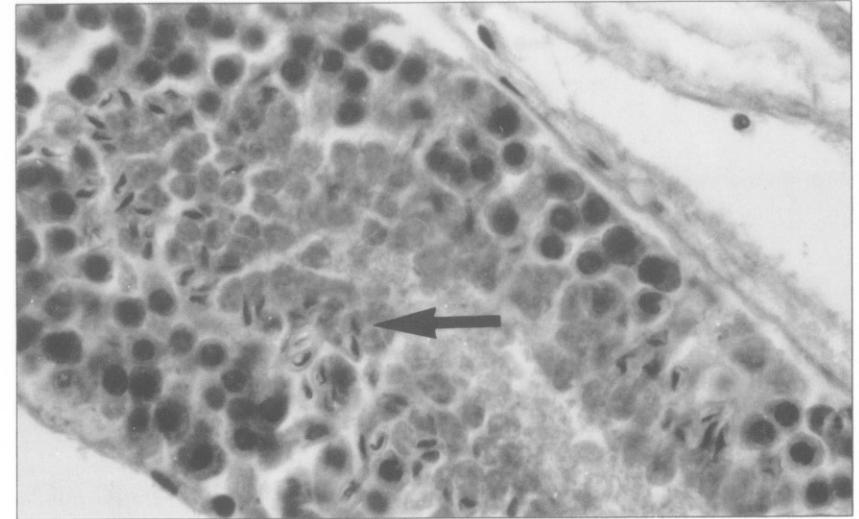


Fig. 4. Cross section ( $\times 400$ ) of a testis from a male recovered in October 1992. Small arrows point to spermatogonia. Large arrows identify primary spermatocytes in the pachytene stage. The asterisk shows chromatin of a dividing primary spermatocyte. A spermatid can also be seen toward the center of the tubule (arrow point).



**Fig. 5.** (Top) An active testis ( $\times 400$ ) from a male recovered in March 1993. The large lumen is bordered by spermatids (*small arrow*) and sperm (*arrow point*). Primary spermatocytes in pachytene stage are identified (*large arrow*). Sertoli cells are also visible (*asterisk*). (Bottom) Cross section ( $\times 160$ ) of the vas deferens in the same male as above showing that mature sperm are stored (*arrow*).



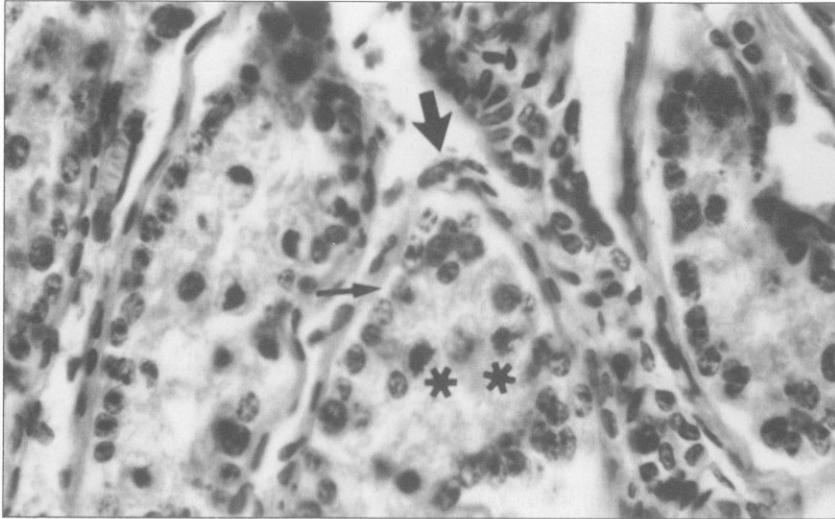
**Fig. 6.** Cross section ( $\times 160$ ) of the testis of a male recovered in June 1992. Mature sperm are visible within the lumen (*arrow*).

July are much reduced, and no secondary spermatogenic elements are seen. A number of primary spermatocytes, however, mainly in zygotene, are observed in males in both July (Fig. 7) and August (Fig. 8).

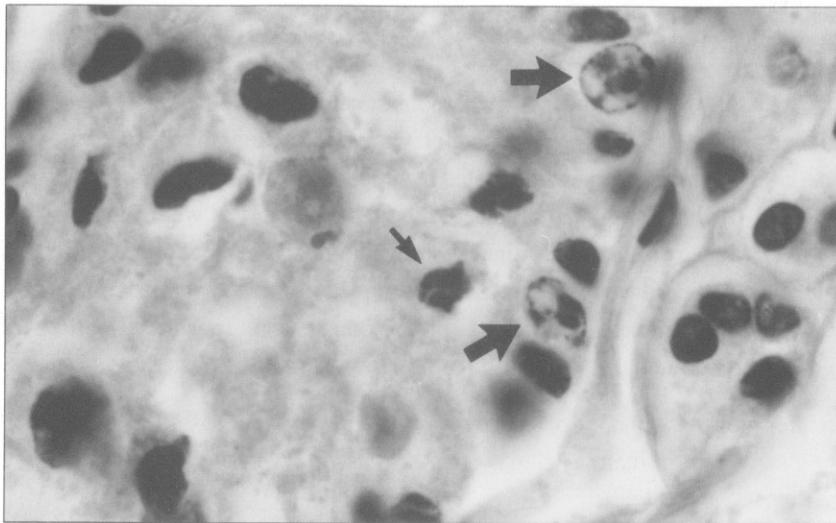
Sexual activity of female Abert's squirrels can be deduced from the presence of well developed embryos obtained in June and July 1992 and March 1993 (Fig. 9). Because of their precarious state of decomposition, the embryos were immediately preserved in alcohol. Crown-rump lengths were determined at a later date. Lactation was indicated by nipples being surrounded by conspicuous and partially depilated areolae. Although no embryos were found in carcasses of June and July 1993, nine lactating females were noted. The contiguous evidence gathered from 1992 and 1993 samples suggests that Abert's squirrels can undergo two gestation periods in any one season. These dual periods of gestation are made possible by the prolonged spermatogenic activity of males.

## Discussion

Tight synchrony between the sexual cycles of male and female Abert's squirrels does not seem to be a prerequisite for two periods of gestation within the same season. Instead, the prolonged spermatogenic activity of males ensures



**Fig. 7.** Regressed testis from a male recovered in July 1992. Tubules are collapsed, and no lumen is present. Leydig cell clumps are small and dispersed (*large arrow*). Numerous spermatogonia are visible (*small arrow*) and chromatin from a dividing primary spermatocyte is identified (*asterisk*).



**Fig. 8.** Regressed testis ( $\times 1,000$ ) in the same male as in Fig. 7. Zygote cells (*small arrows*) and Sertoli cells (*large arrows*) are visible.



**Fig. 9.** A representative embryo from a female recovered in March 1993.

the likely insemination of females at most times of the year. Based on previous approximations of the length of gestation in Abert's squirrels (38–46 days; Keith 1965), we estimate that some females may have been inseminated as early as mid-February.

In this paper, we reconstruct the reproductive cycle of male Abert's squirrels from testicular tissues obtained monthly. Similarly, the female cycle was deduced from the presence of embryos or of lactating nipples. Active spermatogenesis is maintained from October to June. Similar results are seen in the grey squirrel (*Sciurus carolinensis*; Dubock 1979) and in the fox squirrel (*Sciurus niger*; Kirkpatrick 1955). Testicular regression is initiated suddenly in July and is of short duration. Recrudescence of the testes is already evident by October as germinal epithelia resume active meiotic divisions and spermatogenesis.

Males maintain a prolonged regimen of spermatogenesis even during the winter months. This reproductive strategy is presumably sustained in an effort to keep males in mating readiness. Energy to maintain this extensive cellular activity is probably provided by their use of alternative food sources such as the inner bark of clipped tree branches and hypogeous fungi (States et al. 1988). Even when testicular regression ensues, primary spermatogenic elements are still retained. This is contrary to the hamster (Cricetidae) where the epithelium

regresses to Sertoli cells and premeiotic spermatogonia (Bergman 1987) thus ceasing spermatogenesis completely. We refer to this residual spermatogenic activity in Abert's squirrels as maintenance meiosis. In times of need, full spermatogenesis can be rapidly recovered by merely completing the second meiotic division and generating the needed spermatozoa, obviating the need to start from premeiotic spermatogonia. The prospect of early insemination during February is a condition that demands rapid sexual preparedness by the males.

A bimodal spermatogenic pattern has been described for the grey squirrel in England (Dubock 1979) where peaks of testicular activity (weights) are separated by periods of recrudescence. These peaks coincide with litter production during March–April and July–August, respectively. This pattern is not, however, consistent over a 5-year period. In some years, a single, prolonged, spermatogenic-activity peak is observed. Hence, the consistency in reproductive pattern exhibited by male Abert's squirrels will have to be ascertained by continued sampling and analysis of additional collections.

The two gestation periods we observed in females coincide well with those published for the red-bellied tree squirrel (*Callosciurus erythraeus*; T'sui et al. 1982) in which the authors also reported two active reproductive periods—one from January to March and the other from June to August. A similar dual reproduction in Abert's squirrels has previously been suggested (Gurnell 1987) as occurring only under favorable conditions of weather (Heany 1984) and food availability (Gurnell 1987). Neither Brown (1984) nor Nash and Seaman (1977), however, support the hypothesis that individual Abert's squirrels can have two litters in a year. The possibility exists that the two periods of gestation we observed may be transient and characteristic only of certain years when conditions are appropriate. Our evidence of two periods of gestation in 2 consecutive years, however, strongly suggests that the phenomenon may not be unusual.

The inherent mechanism behind the reproductive strategy of Abert's squirrels on the Colorado Plateau may embrace three assumptions. The first assumes that males are consistent from year to year in having a prolonged period of sexual activity extending for most of the year. In this instance, females could control the incidence of gestation by regulating their own cycle according to environmental cues. This female-driven system would, therefore, be instrumental in regulating the yearly frequency of gestation. The second presumes the reverse situation in which females are consistent while males respond to environmental stimuli. In this male-driven system, economy and control of reproduction would be vested in male Abert's squirrels. This contention may be

supported by evidence for weather-induced delays in male sexual preparedness reported by Webley and Johnson (1983). The third strategy suggests that both sexes synchronize their reproduction in terms of fluctuating environmental circumstances.

Our data are more consistent with the first strategy because of the prolonged spermatogenic activity in male squirrels. Hence, the two periods of gestation we observed in 1992 and 1993 could have been the consequence of environmental circumstances favorable to females in promoting timely ovulation and subsequent pregnancies. The decisive pattern (or patterns) existing in Abert's squirrels is uncertain as yet, however, and will require further observations and data. An initial attempt to correlate environmental parameters such as snow, temperature, and total moisture with the dual gestation in Abert's squirrels has not identified a single factor responsible for this reproductive pattern. Dual gestation could be implemented by the animals when climatic factors (such as snow cover) are minimal and temperature is mild (Stephenson and Brown 1980). Reproduction could be limited to one period of gestation when environmental conditions are severe. Similarly, ovulate cone productivity and availability (Allred et al. 1994) could be factors in either promoting or discouraging a biannual reproductive pattern.

Our data do not suggest that individual females go through two gestations in a season. The possibility exists that distinct groups of females become pregnant in winter and in spring, depending on their respective sexual maturity. Similarly, the consistency in the yearly frequency of gestations in females represents a fundamental population parameter that needs to be resolved. These questions are important because they will likely affect management practices related to hunting of Abert's squirrels. Recommendations for habitat management will also be influenced by the frequency of reproduction in these squirrels. The inherent capability of these squirrels to breed either once or twice a year may, in fact, represent an effective means of regulating their population density under differing environmental conditions.

Histological results obtained from animals killed on the road must be interpreted with extreme care because of the postmortem necrosis of the tissues. At present, there are no standards against which to assess these postmortem changes. We have therefore initiated a detailed histological analysis in mice of the effects of necrosis on the testicular histology after death (Pogany and Allred, in preparation). Our preliminary data suggest that the integrity of the seminiferous epithelium is remarkably stable to 12 h after death. Obvious necrotic

changes are only detectable 24 h after euthanasia. The analysis of similar effects on ovaries is presently being undertaken (Pogany and Allred, in preparation). We ultimately hope to provide a scale of injuries (in mice) against which the squirrel histological data can be interpreted.

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