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ABSTRACT: From the fall of 1977 through late spring of 1979, periodic examination of female ground squirrels in the low oak woodlands of southern Tulare County revealed that as much as 20 percent of the reproductively active females bred a second time within a given breeding season. This began to occur 50 to 80 days after the beginning of the breeding season. Evidence of litter loss from abortion was inapparent in 1979, but grossly obvious uterine inflammation was seen in 2 percent of the females in 1978. Neonatal losses were undetermined. Rebreeding appeared to occur in the older females, 2 years and older, and considering that older females probably constitute 35 percent of the breeding females, 20 percent breed-back would seem to be quite significant.

INTRODUCTION

The possibility of second litters in the California ground squirrel has been proposed or theorized by various natural scientists from time to time including Dixon, Evans and Storer. We have tried to develop a reasonable set of criteria to establish this fact using our observations and the comprehensive reproductive work of Tomich for guidelines and basic background material. We have tried where possible to substantiate our findings photographically. Hopefully this evidence will stimulate interest and result in a more thorough study. Our study was prompted because the area has continually presented a serious control problem which only the most zealous efforts by vertebrate pest personnel working with concerned ranchers have been able to demonstrate appreciable success. It is an area in which summer and early fall emergence has been reported time and again and the question of second litters continually arises.

STUDY AREA

The study site was located in the Coho Creek area of southern Tulare County. It was comprised of several sections of low oak woodland at 1,100 to 1,300 feet elevation on the western slope of the Sierra Nevada mountains. Dispersed throughout the easy rolling wooded hills were sweeping open grassland flats and draws with heavy squirrel populations. No control measures had been taken in the main study area the year prior to the study nor the two years during the study.

PERIOD OF THE STUDY

This study was initiated in October of 1977 after two years of severe drought in the San Joaquin Valley and continued through the extremely wet year of 1978 and concluded in April of 1979. The winter of 1979 could be considered a more moderate rainfall year.

METHODOLOGY

Squirrels were taken by .22 cal. rifle for both seasons. The sample size per shooting period in the 1977-1978 season was about 40 squirrels to yield approximately 20 females per period. A thirty day interval was maintained between periods for most of the season. In that season squirrels were taken from a half section area of the study site.

During the 1977-1978 season, squirrels were weighed with a hand held spring scale which was tested to be accurate within 5 grams. External features such as mammary or scrotal development were noted and cheek pouches were checked. Each squirrel was then opened and the reproductive organs were measured and the state of their development noted along with photographing the organs when changes were taking place. Fat stores were assessed and fat classification as described by Tomich was made. A cursory check of major organs was made for gross pathological changes and one tissue sample was submitted for histopathological examination. The stomach was then opened and predominant dietary trends were noted.

During the 1978-1979 season, only reproductive data were collected. The shooting interval was dropped to 10 to 14 days and approximately 20 squirrels or at least 10 females were taken per period. The study area was increased to several sections.

RESULTS

In the 1977-1978 season dietary trends and weights were given only casual attention, but were consistent with the findings of Fitch for the Fisher ground squirrel. Fat storage curves were similar to those given by Tomich for the Beechey ground squirrel except in the fall of 1977 when fat stores were lower than anticipated. This was possibly related to the two years of drought preceding the study. Pathological findings were limited to the fall of 1977 and may have been related to a suspected die-off in the late spring or early summer of 1977. Vector control isolated Pasteurella multocida from one carcass submitted from an area near the study area. In the fall of 1977, we submitted a tissue sample from one of several adult squirrels showing severe liver and kidney involvement and upon histopathologic examination was found to show changes suggestive of plague or tularemia.

Reproductive information was collected for two breeding seasons. In the 1977-1978 breeding season both males and females were studied. Males were already developing quite rapidly in mid-October in the study area. In the north latitudes of the county at the same elevation and habitat, males were still completely regressed. One male in the study area on October 19, 1977 had already attained maximum testicular size with hypertrophied accessory sex glands and the bottom of the scrotum was devoid of hair as if this individual was already sexually active. In general, males in the study area did not reach maximum testicular size until mid or late November and accessory gland hypertrophy did not reach its peak until the first week of December. At the same time as males were showing hypertrophy of accessory glands the older females began to appear with the enlarged turgid tubular uterus of estrus and mammary glands began to develop. Juvenile females, which were showing some uterine vascular change in November, began to show an increase in uterine diameter and mammary development in early December, but were generally behind older females in development.

The 1977-1978 breeding season lasted 120 to 130 days, somewhat longer than that qiven for the Beechey studied by Tomich and the Fisher studied by Fitch. Emergence was first noted the last week of February, 1978 and continued through the first week of June, 1978. That is to say that emergence size young in the 100 to 150 gram range continued to be observed through that entire period, 90 to 100 days, with the bulk appearing the first week in March, but a sizeable number just coming out in late May and early June.

It was noted at about the time of first emergence and in early March that there was a slight drop in embryo count and a more irregular placement of embryos in the uterine horns. Embryo counts for the first 50 days was 9.4, for the next 40 days was 9.7 and last 30 days 7.0. Upon internal examination of the uterus in these late pregnancies there appeared to be fresh placental scars containing readily visible hemosiderin between the embryo sites. This was 75 to 80 days after the beginning of breeding season. Later, lactating females were examined and found to have what appeared to be double sets of primary placental scars. This was not consistent with what we had anticipated for a single litter and was our first evidence of second litters.

Our interest and curiosity had been spurred with these findings because it was not consistent with the findings of Tomich. Though it will be discussed again later, Tomich stated that placental scars could not be utilized in age determination in pregnant uterus. The reason being that he used a translucent light technique where he placed the opened, flattened non-pregnant uterine horns between two glass slides and observed the primary (most recent), secondary and tertiary scars by holding the prepared mount up to the light source. The thickened muscular pregnant horns would not allow trans-lucence. One might assume that primary scars from the previous season had lost most of their hemosiderin by the time of pregnancy, because primary scars were not noted during pregnancy. They definitely were devoid of hemosiderin as secondary scars. So our objective for the second breeding season was to follow the fading of hemosiderin from primary scars as breeding season approached and note whether it had been resorbed by the time of first pregnancies.

When shooting was started in October of 1978 for the 1978-1979 breeding season, each uterine horn was opened and all changes were noted. It is interesting that breeding season started three weeks later this year and lasted 90 to 100 days with a proportionately shorter emergence period.

Adult females in estrus were not seen until January 3, 1979; hemosiderin was not observable to the naked eye. Grown junvenile females were developing, but were about two weeks behind the older females. By January 15, 1979 pregnant adults were found with 7 to 21 day embryos. Each pregnant horn was opened and the space between implantation sites was clean and devoid of any grossly visible hemosiderin. Three older females were taken at the same time which were in or near estrus. Two showed no hemosiderin and one showed traces of hemosiderin or hyperemia at the old primary scar sites. Young females were coming into estrus for the first time around January 15, 1979, but could be distinguished from the older females for the most part by lack of uterine musculature and generally smaller mammary development. From this point on, it was usually impossible for us to distinguish between old and young females by casual observance.

We continued to open every pregnant uterus in each succeeding period. About 50 days after the beginning of breeding season, we began to see the appearance of fresh primary placental scars between embryo implantation sites. They proved to occur in 20 percent of the bred females observed. As lactating females began to appear above ground, fresh primary scars in each horn were counted. The occurrence of double primary scars then appeared and breeding dates for these extrapolated back to when we first saw primary scars in pregnant females. Shooting periods were dropped in late spring due to other commitments before an accurate percentage could be established in the lactating and the weaned portion of the females with double primary scars. It is also a difficult time to get good representative numbers of this group because they gain rapidly and feed less frequently and samples are comprised mostly of larger juveniles of the year. Evidence from the previous season indicated that many of the older females who were active during late spring and summer had double primary scar counts.

Embryo resorptions were noted in both seasons and occurred mostly in the first third of the breeding season. In the 1977-1978 season, it dropped the mean embryo count from 9.7 to 9.4 embryos per females. In the 1978-1979 season, resorptions accounted for a drop in embryo count from 9.2 to 8.5 embryos per female. These resorptions appeared to be occurring mainly in older females. While a few resorptions could be found throughout the season the smallest incidence was during the mid-season peak when most pregnancies were of young females.

Evidence of uterine inflammation which might indicate the possibility of abortion occurred in only 2 percent of all breeding females in the 1977-1978 season. No abnormalities were seen in the 1978-1979 season.

The data thus far collected are the result of eighteen months of shooting and 625 squirrels were taken, 575 in the study area and 50 from a more northern location for comparison. Also data from routine shootings by vertebrate pest inspectors on 135 squirrels taken throughout other county foothill locations were checked on a casual basis to better understand the extent of reproductive variation in this county.

DISCUSSION

It has been known for some time that the breeding season for the Fisher ground squirrel in the southern San Joaquin Valley is somewhat earlier than in the northern half of the valley. Storer in surveying U.S. Public Health Service records noted that the Fisher ground squirrel bred from January to as late as June in some instances with some summer and early autumn emergence. In another survey he noted that squirrels in Los Angeles County bred from the first of December on into June with a few litters (0.1 to 0.6% of the total active population) from July through October with no pregnant females taken in November. In Fitch's study in Madera County in the low oak woodland breeding season was in January and February with two to three weeks variation from year to year. He felt two months was essentially the average length of active breeding. Storer and Evans studying the Fisher ground squirrel a few miles east of Fitch's study, but at 4,500 feet elevation found breeding season to be April and May.

In our study area in southern Tulare County, the 1977-1978 breeding season was from early December through the first week of April or approximately 120 to 130 days with the greatest breeding activity being 90 to 100 days. Emergence began the last week of February and continued through the first week of June or 90 to 100 days. This was considerably longer than anticipated and in lieu of our suspicions of second litters, this was believed to be quite significant. The 1978-1979 season on the other hand did not start until three weeks later than the previous season, but ended at approximately the same time shortening the emergence period proportionately.

In relationship to the Beechey ground squirrel as studied by Evans, Lindale and Tomich, breeding season began somewhat later, usually in February, March or April. The breeding season lasted 85 to 113 days with the main breeding activity occurring in 80 days.

The actual breeding curves for the Fisher and Beechey ground squirrels are similar in appearance. The diphasic curve has a main peak, then tapers off to a lower plateau and then drops off again. The curve for our second breeding season established from 10 to 14 days samples showed two distinct peaks, a larger initial peak and a smaller secondary peak. Dixon believed this diphasic curve was possibly an indication of second litters of females which lost their first litters. Evans found a similar diphasic curve in Alameda County and referred to Dixon's theory as a possible cause.

Tomich on the other hand believed the diphasic curve was a reflection of the seasonally polyestrus nature of the Beechey. He believed that late breedings were associated with squirrels who did not conceive on the first estrus, but did conceive on a later cycle. The peak of breeding during the second phase was 30 to 45 days following the first. This seems a little long for a single estrus cycle, but may actually be the case.

Be that as it may, we feel that something different is occurring in the Fisher ground squirrel. In the Fisher the diphasic curve seems to be a reflection of a second litter as postulated by Dixon and not simply delayed conceptions. Criteria have been established which we believe substantiates this.

To reiterate, Tomich states that placental scars are plainly visible from one season to the next differentiating primary, secondary and tertiary et cetera, primary scars being the most recent. While this is the case, he also stated that in pregnant females the placental scars could not be used for age determination because they could not be seen in the thickened muscular walls. Then following birth the new or primary scars were readily visible red stained areas while secondary scars were seen by the assistance of transluscent light because remnants appear as small white or pink obscurities in the thin non-pregnant wall and not readily visible to the naked eye.

We believed this was an important point in that hemosiderin, the breakdown product of hemoglobin at the site of the scar must decrease through the season, being resorbed by the time of pregnancy the following year. Lindale had previously noted that it decreased through the summer months. As the female conies back into estrus the following year, we found that the staining has been almost completely resorbed and is inapparent to the naked eye in the early pregnant older females. Therefore the appearance of fresh scars between embryo sites in late litters would indicate that they are primary in nature and represent an earlier litter of the same season.

Now to determine which faction of the female population is involved and to what extent is more difficult. Fitch and others have noted that the older females breed several weeks ahead of yearling females. Our findings were consistent with this. Evidence of breed back appeared to occur 50 to 80 days after the beginning of breeding season. In following the active females, it was believed that older females bred back after yearling females peaked out. The first season of our study we had some emergence before discovering that some females had bred back, but it does not appear that weaning is necessary before rebreeding. The timing of breed back in the second season of the study was such that

young could only have been in the 3 to 5 week age range and still dependent on their dams. Of course much of this is speculative because once young females became pregnant we did not attempt to distinguish them from older females.

The question arises as to whether the females suckling young are breeding back or whether the young are lost from abortion or neonatal death with females subsequently breeding back. Approximately 20 percent of the pregnant females examined showed evidence of a second breeding. This is a substantial percentage. Gross evidence of infection and therefore the possibility of abortions was found in only 2 percent the first year and none the second year. Abortions from stress if they exist in the natural state and neonatal losses could not be determined. If one uses population statistics gathered by Fitch on the Fisher ground squirrel, the older females comprise approximately 35 percent of the breeding females. That would mean that about 57 percent of the older females were breeding back (20 out of 35, assuming the 20 are older females). It would seem that this is rather high to be attributed to abortion or litter loss on a reoccurring annual basis; yet if rebreeding is a normal phenomenon of older females, why then is it not a higher percentage?

The possibility of the Fisher ground squirrel having a normally occurring two litter cycle should not be unequivically dismissed. It is known that the eastern fox squirrel will have one litter as a yearling and two litters a year as an adult. Also it is known that many rodent species can breed back while having given birth only a few hours before breeding, Microtus and Sigmodon being good examples of this. It is also stated by Tomich (referring to Johnson) that the thirteen-lined ground squirrel, Citellus tridecemlineatus is believed to be an induced ovulator with some individuals reportedly having second litters.

One might also consider the environment as an important contributing factor in relationship to two litters. Favorable warm inversions in the fall and winter coupled with a longer photoperiod and the heat moderating effects of tree cover in spring and summer may play a role in the reproductive cycle in this area.

CONCLUSION

Evidence submitted here on second litters on the Fisher ground squirrel should not be considered conclusive, but should stimulate renewed interest in the breeding cycle of the Fisher ground squirrel, particularly in its southern sheltered ranges which are ideal for long periods of activity. It appears that a significant number of second litters is occurring annually whether related to early loss of the first litter or as a normal aspect of the reproductive cycle.

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