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Age Determination of the Cotton Rat *(Sigmodon hispidus)**

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I. Abstract

A study based on 316 known-age specimens of the cotton rat, *Sigmodon hispidus hispidus*, from southeastern Louisiana consisted of a laboratory and a field study. The categories of morphological characters examined were: body measurements; pelage and molting; reproductive activity; teeth; skeletal growth; and lens weight. The changes of these characters were studied through twelve months of age. The laboratory study showed that body length, molting stage, epiphyseal fusion, skull measurements, and dry lens weight combine to give a high degree of success for age-determination through six months of age.

The field study consisted of releasing 96 known-age cotton rats and successive periods of retrapping. The retrapping data support the conclusions of the laboratory study. Weight as an age-determining character is discussed and evaluated from the field data of this and other studies.

* This paper is based on a dissertation submitted in partial fulfillment of the requirements for the Ph.D. degree in Zoology at Tulane University, New Orleans, Louisiana, 1963.

II. Introduction

The need for reliable criteria for estimating the age of wild animals has been noted by many researchers and has been reviewed by Alexander (1958). Support for the study of age-indicating techniques has been devoted primarily to the larger game mammals due to sporting interests. Even when studies have been undertaken on smaller mammals, the emphasis is still placed on those species that are of interest either for hunting or for their commercial fur value. With the recent interest in the study of populations of small rodents and particularly the occasional dramatic fluctuations in their numbers, there is an increasing need for accurate methods for determining age classes.

The degree of accuracy necessary for estimating the age of any given animal will depend on the species itself. For example, if the species does not breed until it is several years old, the estimation of age in units of time of less than one year will have little practical value. If, however, the species breeds at the end of its first year, then estimation of age to one year or even less would be desirable to distinguish between young of the year and adults for the next breeding season. In the case of small rodents more precise estimation of age is necessary as many of the young will become reproductively active during the breeding season in which they were born. Three or more generations in a breeding season of a small rodent are not uncommon and may be the rule especially if the species breeds throughout the year. Occasional trapping in the animal's natural habitat will not solve the problem of age determination as the age of the captured animals so collected will be unknown, and only rough approximations of age classes can be formulated. A solution can only be found by first studying the development of various morphological characters on animals whose individual ages are known. Ideally, field studies should be undertaken concurrently on known-age animals to verify or adjust the laboratory derived data.

Thus, the purpose of this study was to determine useful age-determination characteristics from laboratory-raised cotton rats *(Sigmodon hispidus)* and to field-test the application of those techniques. Hopefully, the techniques that are successful for determining the age of the cotton rat will aid other researchers in age determination of small rodents.

Studies on growth of the cotton rat are limited largely to weight and measurement data. Svihla (1929) described cotton rats at birth, their early growth, and their ability to feed at about ten days of age. Meyer and Marsh (1943) noted that cotton rats become sexually mature at six to seven weeks. In the most elaborate study of growth of the cotton rat Meyer and Meyer (1944) presented considerable data on the growth of the young and additional data on growth through 15 months. Body weights are given in units of 50 days of age, but do not give good indication of being reliable criteria for aging of the animal. Furthermore, the sample size is limited to three to six animals. Information was presented that the rate of growth is influenced by the age of weaning. Data were also given for growth of various endocrine glands, but the areas of overlap from one age group to another were too extensive for estimating the age. No data were presented on body measurements, the growth of bones, the progression of molts, the development of the baculum, or the several other categories investigated in this study.

McIntire, Schweigert, and Elvehjem (1944) studied the increase in weight of the cotton rat to six weeks of age. The authors noted that both sexes grow at the same rate although the female is somewhat smaller than the male at any given age. Odum (1955) indicated breeding begins at a weight of 62-87 grams and at an age of 40-50 days. Individual size of the animals varied with the density of the population, animals being larger at lower than at higher densities. Data on weight were given for a limited number of known-age individuals. Odum also noted that very few individuals survived six months of live trapping, which may give some indication of longevity under field conditions.

Sealander and Walker (1955) suggested that size is no indication of reproductive capabilities. The population studied by these authors was aged by the use of the laboratory weight data of Meyer and Meyer (1944). Age classes based on body measurements were thought to be of little value as these classes had little relationship to weight. Keys (1958) followed the rate of embryonic and early postnatal development. He noted that

the cotton rat is quite precocious at birth and develops at a remarkably rapid rate. His study was not carried beyond 15 days of age.

III. Material and Methods

A. Laboratory Study

A breeding colony of wild cotton rats from southern Louisiana was organized in the spring and summer of 1960. Some of the young born of the wild cotton rats were also used as breeders in the laboratory. Breeding pairs were housed in large cages. Large fruit juice cans and cotton batting were found to make satisfactory nesting sites. Little fighting occurred after the first few days of pairing.

The females usually bore young two to four months after pairing. The male ignored the newborn young, and was left continually in the cage to take advantage of the post-partum estrus. The breeding females were palpated every two weeks to determine pregnancies. Once a litter was expected the cage was checked daily. At parturition the female would bar the male from the nest can; he then made a nest elsewhere in the cage. This action normally served to establish the date of birth. Also, as the eyes of the young are closed at birth but open the next day, a quick check of the newborn young would verify the date of birth.

The laboratory-born young that were used as breeders were usually paired when weaned (three weeks). At this age males and females were amicable. If pairing was delayed until after six weeks, some fighting resulted. In a few instances when the newborn young were a week to ten days old, the female, whether wild or laboratory raised, would viciously attack the male, chewing the hide from the back of the head to the sacral region. The pair was then separated and no further attempt was made to breed the female, although invariably a litter was born about two weeks after the separation.

The cotton rats were fed a diet of Wayne Lablox exclusively. Water was available *ad libitum*. The breeders were occasionally fed fresh greens in the earlier stages of this study. Most of the laboratory-bred cotton rats were secretive during the day, or at night when any activity was taking place in the laboratory. Although I made constant efforts to handle and tame the cotton rats, especially the young, the attempt proved useless. The tamest animals in the colony were wild-born, adult breeders. Generally the animals were removed from their cages by trapping them in their nest can or by chasing them into a Sherman live trap. The animals were then etherized for examination. Once subdued the animals would not bite but would still try to escape.

Meyer and Meyer (1944) showed that cotton rats weaned at 10 days of age did not gain weight as rapidly as those weaned at 20 or 30 days, the difference between the latter two ages being slight. Rabasa (1952) showed growth rates in albino rats to vary depending on the number of individuals per cage. For these reasons all cotton rats were weaned at the same age (21 days) and, with the exception of the few used for breeding purposes, were caged individually. Cages contained between 110 and 120 square inches of floor space and were supplied with coarse sawdust or shavings, a nest can of suitable size, and some cotton for nesting material. The nesting material was extensively used during winter, but was generally ignored during summer, although the temperature in the air-conditioned animal room was seldom lower than 65° F or higher than 75° F. I attempted to limit the photoperiod to that available as sunlight. Occasionally the animals received additional light when night work was necessary. The variations of light and temperature were kept to a minimum whenever possible, but may have affected some of the laboratory data, as will be discussed below.

The study was based on 12 samples. The first sample was made up of one-month-old animals. Successive samples were one month older than previous samples through 12 months of age. Each sample was derived from 3 to 5 separate litters and was planned to be composed of 5 males and 5 females. Cotton rats were assigned at the time of weaning to the older incomplete month-age samples. The first few litters were composed predominantly of males, causing the 12-month sample to have an undesirably high number of this sex. With the exception of the conditions described above, the animals were assigned to a sample at random. A few individuals died or were accidently killed prior to attaining their desired age, causing some deviation from this plan. Adjustments were made where possible.

Three wild-caught individuals were kept

TABLE 1.
Distribution of individuals forming the laboratory study.

	Age of Sample (Months)												
	1	2	3	4	5	6	7	8	9	10	11	12	18
Males	5	5	6	5	5	5	5	6	3	3	4	9	2
Females	6	5	6	5	4	5	8	1	7	6	5	2	1
Total	11	10	12	10	9	10	13	7	10	9	9	11	3
Number of litters represented	4	5	5	5	4	4	5	3	3	3	4	4	–

in captivity as breeders for 16 months. These were judged to be two or more months old at capture and are thus considered to have been 18 months or older when sacrificed. The data from these individuals were incorporated where possible. The composition of each month-age sample as to number, sexes, etc., is given in Table 1. The animals used in this study came from litters ranging from 5 to 10 young. No significant difference existed in weight or body measurements between the different sized litters.

All cotton rats were etherized and examined every two to four weeks for molting and reproductive condition. Individuals were sacrificed with ether when they had reached the desired age in months and on the same day of the month as their date of birth. The animal was then weighed, and the standard body measurements [total, tail, hind foot (not including nail), and ear (measured from the notch) lengths] were taken. The cotton rat was then skinned, the pelt being pinned out flat for drying. Both eyes were removed, placed in formalin, and the lenses later removed. The lenses were dried and weighed on a Mettler analytical balance to 0.1 mg.

The skull was severed, dried, and cleaned by dermestid beetles. The left forelimb and scapula were removed, fixed in 40% isopropyl alcohol, and later macerated and stained to study the epiphyseal areas. The right forelimb was similarly removed and preserved in formalin together with the tail. Both structures were later X-rayed for epiphyseal fusion. The entire penis was removed, macerated, and the baculum stained. Other reproductive structures were inspected, and pertinent notes taken. The remaining portion of the carcass was preserved in formalin.

B. Field Study

Growth data on laboratory-raised individuals of a wild species would not be expected to be the same as on animals in their natural habitat. Many differences exist between the laboratory and the natural habitat, such as food and climatic factors. Moreover, the cage environment may produce conditions that affect the normal growth of the animal. The limitations of activity, the lack of extreme environmental variation, and the change in social patterns are difficult to evaluate. Thus I attempted to obtain growth data on known-age cotton rats in their natural habitat by releasing and retrapping young cotton rats born in the laboratory (individually marked by toe-clip). The retrapped individuals were examined and released, hopefully to be retrapped a second time.

IV. RESULTS

A. General Body Measurements

The body length measurement was obtained by subtracting the tail length from the total length. I consider this computed measurement more reliable than either of the other two measurements, as portions of the tail are frequently lost.

The data for weight (Fig. 1) and for body

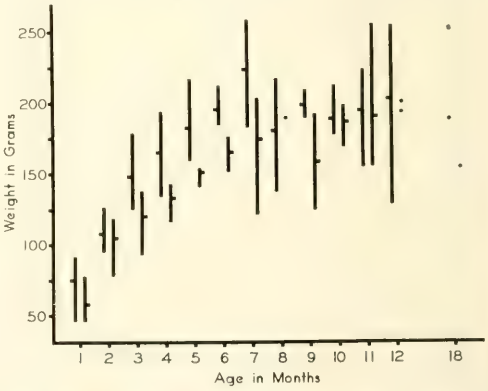

Figure 1. Body weight when sacrificed of known-age cotton rats by sex. The vertical line indicates the range and the horizontal line the mean of the sample. The left line of each pair is the data for males, the right line the data for females.

length (Fig. 2) are presented by sex. The difference between the sexes for these characters was considered significant ($P \leq 0.02$). The sexual dimorphism of hind foot and ear length (Fig. 3) is not considered significant ($P \geq 0.3$), and the data are combined for both sexes.

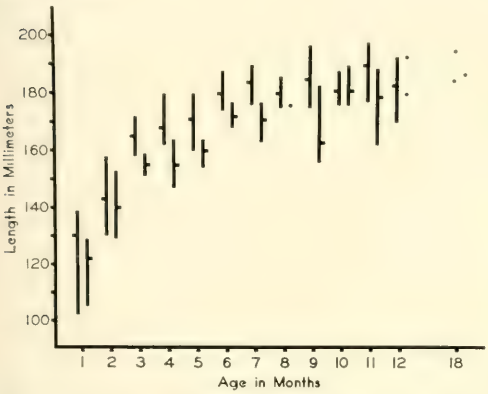

Figure 2. Body length when sacrificed of known-age cotton rats by sex. (See Figure 1 for description).

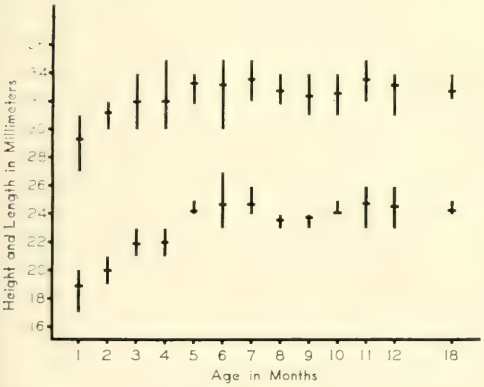

Figure 3. Ear height and hind foot length when sacrificed of known-age cotton rats. Data are for both sexes. Upper series of data are for hind foot length; lower series of data are for ear height. The vertical line represents the range and the horizontal line the mean of the sample.

While of limited value for age determination, these data should be considered in detail as these measurements are classical mammalian measurements. The measurements differentiate satisfactorily between one- and two-month-old animals, and distinguish these two age groups from older specimens. The age groups are best separated on the basis of body length and, to a lesser extent, by hind foot and ear lengths. Weight appears to be least related to age and is the most variable measurement.

The usefulness of any character for age determination depends in part on knowing and evaluating its variability. One possible variable might be the day length during the first few months of the cotton rat's life, since this was not controlled. The body length of nine males and five females born between September 8th and 12th are compared with 11 males and 12 females born between January 11th and 14th (Fig. 4). The first group

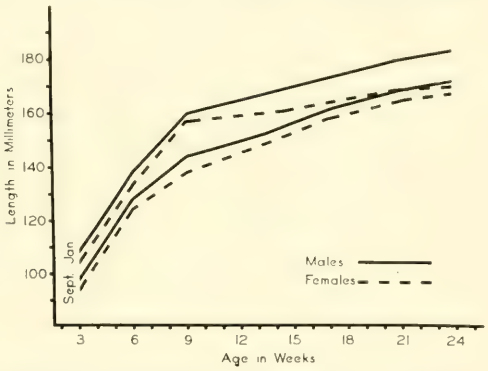

Figure 4. Variation in mean growth rates of known-age cotton rats, depending on the time of the year in which the animals were born.

was exposed therefore to a decreasing day length for three months and then to an increasing photoperiod, the second group being exposed only to an increasing day length. The January group was consistently larger at each age in body length than the September group. While Figure 4 shows only the means for the sample, the ranges of the measurement showed little overlap. At three weeks of age the means differ by 10 mm, while at nine weeks the differences are 14 mm for females and 19 mm for males. At these two points there are no areas of overlap of the range. For the males this difference in body length is maintained until twenty-three weeks of age, the limit of the data. For the females the means appear to be approaching each other at this age.

Body length is the most reliable measurement for age determination (Fig. 2). However, the seasonal variation, whatever its basis, could handicap the data. Perhaps separate growth curves could be used during different seasons, but this seems a questionable procedure.

A second source of variation might be

genetic, i.e., would growth curves determined by animals from one area be applicable to animals from another area? The only other study of laboratory cotton rats is that of Meyer and Meyer (1944). The stock for their colony came from Baton Rouge, Louisiana, 80 miles from the locality of my stock, and belong to the same subspecies *(S. h. hispidus)*. The only comparable data presented by these authors are body weights, and when their growth curves are plotted with mine the curves are virtually contiguous. This fact does not really answer the question posed as to genetic variability, although the similarity of the two growth curves suggests the data of my study might be used within the range of this subspecies safely.

A third question raised by the data is the apparent decrease in means and ranges of measurements at 8 or 9 months in Figures 1, 2, and 3. Each age sample comprises only the measurements of the sacrificed animals. Thus at 8 or 9 months there is no decrease but only the data from smaller animals. The reason for these smaller animals is not at all clear. The best suggestion seems to be the season in which the specific individuals were born. Those animals forming the eight- and nine-month samples were born in November and December, while those forming the younger age samples were born in January through March. Photoperiod can be suggested as the cause, but the mechanism is only conjecture.

Undoubtedly other factors will affect body weight and measurements. Sealander and Walker (1955) and Dunaway and Kaye (1964) have shown the mean body weights to decline during winter when depot body fat is being rapidly consumed and recruitment of the population is low.

B. *Pelage and Molts*

The results presented in this section are based primarily on observations of the dried pelt, with data of live animals being used as a check and/or elaboration.

The pelage of cotton rats displays a remarkable degree of uniformity throughout the animal's life. All the pelts were viewed at one time, but no real variations in color, shade, or texture could be observed except for very young cotton rats (one to two weeks old) in which the pelage was notably softer and composed of shorter hairs.

The actual molting patterns or progressions are diagramed in Figure 5. Several specific molts can be demonstrated ontogenetically. The newborn cotton rat is completely furred with short hairs somewhat darker than the adult. The juvenile pelage becomes complete within one week of age at which time the young cotton rat begins the very rapid molt to the subadult pelage. This molt begins in the ventral thoracic region. The three-week-old animal is molting laterally. At four weeks of age the animal is molting dorsally only. The new pelage is complete between five and six weeks of age, and the animal is now a subadult (i.e., may become reproductively active but physically smaller than the adult body size). This molt is extremely constant both in pattern and duration.

The next molt is termed the adult molt, since at its completion the cotton rat has reached adult size and the growth rate decreases abruptly. This molt usually begins at five to six weeks of age, often while the previously described molt is still present dorsally particularly on the top of the head. The progression of this adult molt is the same as for the subadult molt except for duration. The first molt requires about one month for completion while the adult molt requires two and one-half to three months. As a practical point, these two molts can be distinguished by: (1) the presence of juvenile pelage dorsally in the subadult molt; (2) the adult molt occurring as a narrow lateral band while the subadubt molt being present over one-half of the body surface at once; (3) the small size of the animal during the subadult molt.

As early as five months but usually around six months, the cotton rats molt again. This molt, very irregular both in duration and extent, has been termed a patch molt. My observations indicate that this molt follows the general pattern described for the previous molts and differs in that only small disconnected areas are molting at any one time. Both the duration and precise progression of this molt are difficult to follow, especially since the animal will frequently stop molting only to start again where it stopped or commence a new molt, or both.

Further adult molts occur with increasing irregularity both in area and duration. Molting is most easily observed when present laterally, particularly around the front limbs

and on the cheeks. The molting areas are small and scattered, and become even more so as the molt progresses dorsally. I have presumed the typical ventral-to-dorsal progression occurs, but definite observations are insufficient for a positive description due to the limited number of animals raised to the older age samples. The later adult molts appear strongly influenced by photoperiod and, in the case of a few known-age females used as breeders, by reproduction as well.

The adult molts need more critical study than could be undertaken. However, if a normal longevity of six to eight months is postulated, then the molting pattern, if closely examined, could be helpful for estimating the age of the cotton rat. If the specimen shows no molting, then the animal is either in complete subadult pelage (about six weeks old) or in complete adult pelage (about five months old). Body length or weight could be used to distinguish these two ages. The use of the molting pattern for age determination of cotton rats does not consider environmental variations.

Photoperiod will potentially influence the use of molting as an aging device, although the major variation seems to occur *after* the subadult-to-adult molt has been completed. Mohn (1958) described the development of "growth waves" (i.e., ontogenic molts) for the black rat *(Rattus norvegicus)* which are virtually identical to my observations in age at onset, progression, and duration. The similarity of these observations as well as those by other authors suggests that the first few molts (to the adult pelage) are more influenced by ontogeny than other factors. Mohn also comments that the later (adult?) molts are considerably more variable and the frequency and rate are retarded by both pregnancy and lactation.

A microscopic examination of individual hairs and groups of hairs was undertaken. The hairs of one-month-old animals were distinctly shorter and were a mixture of the shorter juvenile hairs and the incompletely grown subadult hairs. Otherwise microscopic examination of the hair was of no value for age determination.

C. Reproduction

The condition of the external reproductive structures was included in the periodic ex-

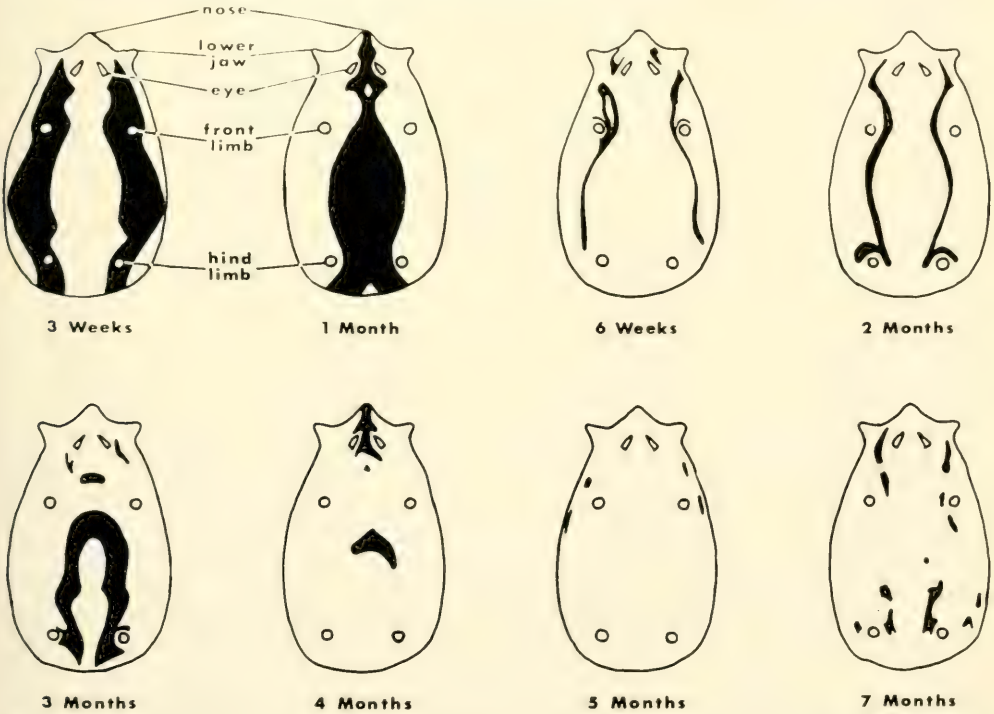

Figure 5. Molt patterns of known-age cotton rats viewed from the skin side of the pelt. Darkened areas indicate areas of active molting.

amination of the live cotton rats. For males the position of the testes (scrotal or abdominal) was determined. The pigmentation and relative size of the teat and the condition of the vaginal orifice (perforate or imperforate) were recorded for each female. Additional data were derived from an examination of the birth dates of litters from known-age parents.

The youngest age at which a rat gave birth was 65 days, having been impregnated by a litter mate. Subtracting a gestation period of 27 days (as determined by Meyer and Meyer, 1944) gives an age at conception of 38 days. This particular individual was the only spring-born (February) female that was used for breeding purposes. All other data on age at first litter are from animals born during September and October. For these individuals the youngest age at which a female gave birth was 84 days, with conception thus occurring at 57 days. The age at conception of the first litter for other females ranged from 70-100 days, with the older age being more common. Meyer and Meyer noted one female being impregnated at 40 days of age and several others by 50 days of age. These authors also noted the first estrus to occur at a younger age during the period January-through-June than during the period September-through-December.

The condition of pregnancy is of value for age determination only to the extent that the investigator can determine a probable minimal age. Furthermore, nonimpregnated females need not necessarily be less than that minimal age. Additionally, Odum (1955) and Haines (1961) have shown that the cotton rat has seasonal reproductive peaks and occasional periods of anestrus, especially during the winter. Presumably photoperiod affects the age at onset of reproductive maturity although other factors undoubtedly exist. Thus pregnancy is at best a limited tool for age determination.

The earliest age at which a perforate vaginal orifice was noticed was six weeks. By three months all females had shown this condition at least once. When sacrificed, one two-month-old female had a distended, fluid-filled uterus, a condition described by Clark (1936) as occurring during proestrus and estrus. An active estrus, as determined by a perforate vaginal orifice or an examination of the uterus, might be more helpful for age determination than pregnancy as it occurs independent of male cotton rats. However, the condition is still influenced by season and, like pregnancy, is of value only for establishing minimal age.

Size and extent of the pigmentation of the teat were constant for females two months or older, except for pregnant and nursing individuals. One-month-old females showed no pigmentation except for the one female mentioned above that bred at 38 days of age. By two months all females showed some pigmentation, and by three months all possessed a dark-brown pigmentation of the teat. A week or ten days prior to the birth of a litter the teats become enlarged and change from dark-brown to black pigmentataion. The dark-brown color returns after weaning if the female is not pregnant. The size and degree of pigmentation of the teat suggest an expression of sexual maturity and activity rather than of age.

Sexual maturity in males can be determined by the presence of viable sperm in the epididymis. No sperm were seen in smears of the epididymis of one month and six-week-old males. The testes normally descend and remain in the scrotal sac at two weeks to one month for laboratory-raised young. Of the five two-month-old males, only two contained sperm in the smeared epididymis. Viable sperm were present in all males three months of age or older. The two earliest pregnancies reported above (conception at 38 days) were by males of the same age as the females. Haines (1961) has shown that male cotton rats vary as much as females seasonally, although I did not notice this. Whatever the variations and their causes, reproductive maturity in males is only indicative of a minimal age of perhaps two months and is thus of very limited value for age determination.

The baculum and its digital processes were studied after maceration and staining. Maceration was considerably hastened by preserving the penis in 40% isopropyl alcohol. Stained specimens were stored in glycerine containing 0.5% phenol to inhibit mold. All bacula were measured with dial calipers. The total length and the height and width of the base were carefully examined, and character indices were attempted. One-month-old animals were distinct, but all older age groups encompassed the same measurements. The staining of the bacula showed a high

degree of uniformity, variations being probably due to staining technique.

Forty-two bacula had digital processes that were satisfactory for study. Of these, 18 were less than seven months of age and showed no deposition of stain in the processes and may thus be assumed to exhibit no ossification. Seven-month-old specimens show a small amount of stain in the tip of the medial process, which increases in extent with age. The lateral processes begin to show staining at nine months, also increasing with age. The extent of the staining is nearly complete at 12 months, showing very little increase at 18 months. The progression of the stained areas is illustrated in Figure 6.

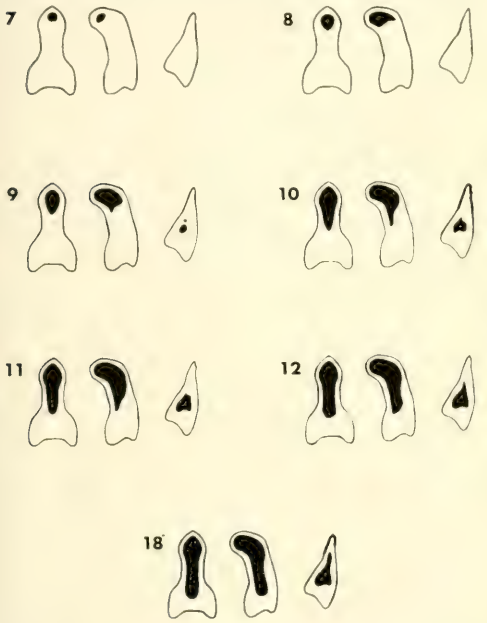

Figure 6. Digital processes from bacula of known-age cotton rats. Number at each group of three indicates the age. The first figure on the left is a ventral view of the median cartilage; the middle figure is a lateral view of the median cartilage; the figure on the right is a ventro-lateral view of a lateral cartilage.

The value for age determination of the staining of the digital processes is limited to male animals over six months of age. In all probability this would be a very small portion of the population. If sufficient numbers of males with ossifying digital processes could be collected, some evaluation of the age composition of the older individuals could be made, especially if one presumes the same age distribution for the females.

D. Teeth

Twelve measurements were made of various individual teeth and tooth rows. The results were effective only to separate one-month-old cotton rats from all older age groups which remained indistinct from each other.

Three subjective tooth characters became evident when measuring the skulls. When viewed laterally, the occlusal surface of the lower molariform teeth appears as a straight line for the younger animals. For the older individuals this view becomes more concave, presumably due to the grinding of the two tooth rows on each other (Fig. 7). Subjective categories with numerical values were applied in an attempt to evaluate this uneven wearing. The technique did not prove successful and was particularly unworkable for very old animals.

The second character was the differential wearing of the first lower molar, the posterior two thirds being ground away more rapidly leaving a short prominence or spike on the anterior third (Fig. 7, C and D). The prominence was categorized numerically but the results were similarly unproductive especially for very old individuals which often lacked the prominence.

The third character was the reentrant angle or groove on the lateral crown surface of the molar teeth. As the tooth is worn away, the groove decreases in length and becomes more exposed. Since the size is too small to measure, I attempted to evaluate the length of the groove as the ratio (expressed as per cent) of this length to the exposed crown height. This evaluation was only made on the first and second molars as the third is partially concealed by the base of the coronoid process. Again the characterization was not significant. The decreased length of the groove is evident in Figure 7.

At the age of one month only eight molars are visible in the cotton rat. The third molar (both mandibular and maxillary) has not yet broken the skin although the tooth is not covered with bone. By two months, the tooth has completely erupted and has attained approximately the same height as the first and second molars.

The occlusal surface is quite constant throughout the life of the cotton rat. The

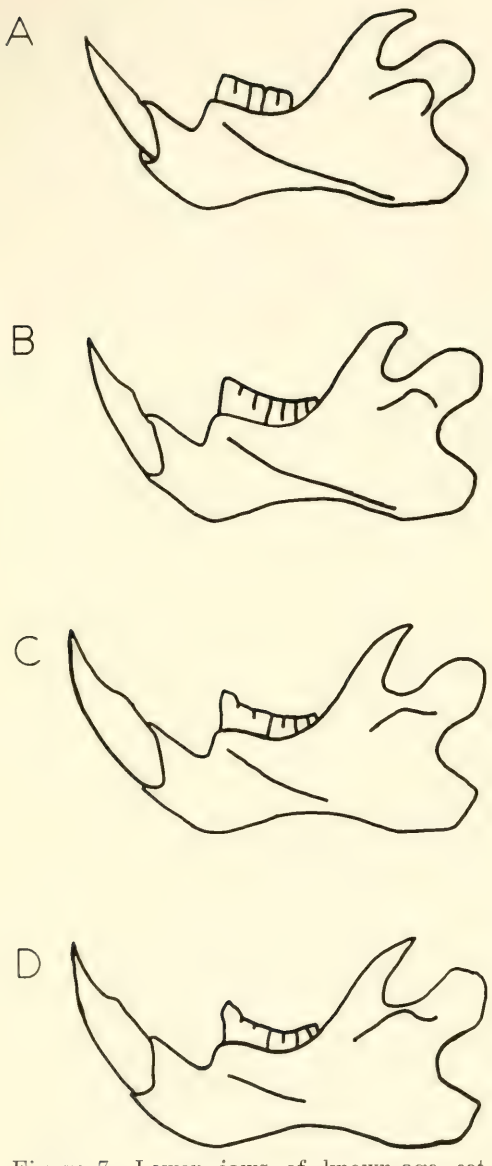

Figure 7. Lower jaws of known-age cotton rats. A—1 month, B—3 months, C—6 months, D—9 months.

changes were more difficult to evaluate than the three subjective categories listed above and thus were of even less value for age determination.

E. Skeleton

Skulls of a male and a female of each age group were randomly selected for a preliminary study of characters that might be useful for age determination, measurements being made with dial calipers. Over thirty measurements were investigated. Measurements of: 1) nasal length; 2) greatest zygomatic breadth; and 3) greatest width of the lambdoidal crest seem to be related to age, and were made on all skulls, as was the condylobasilar length. The data from the four measurements are summarized in Figures 8-11.

For the condylobasilar length (Fig. 8) the data are presented separately for each sex, the males at each age having significantly longer skulls than the females ($P \leq 0.05$ at one month of age; $P \geq 0.02$ at all older ages). For the other three measurements (Figs. 9-11) the differences between the sexes were small although the males were uniformly larger. P values were as low as 0.1 in a few instances but for the most part were 0.3 or greater. The data do not warrant being

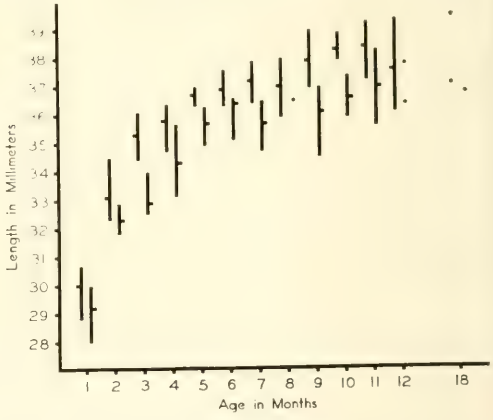

Figure 8. Condylobasilar length of known-age cotton rats by sex. (See Figure 1 for description.)

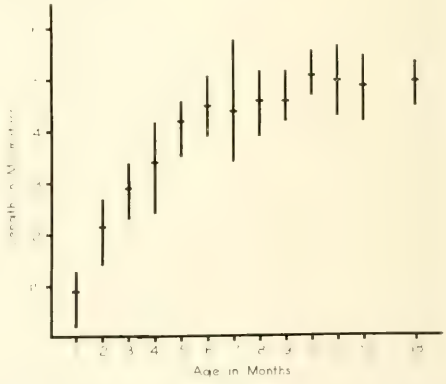

Figure 9. Nasal length of known-age cotton rats. (See Figure 3 for description.)

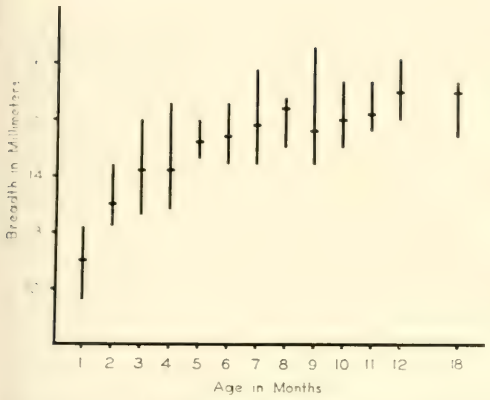

Figure 10. Lambdoidal breadth of known-age cotton rats. (See Figure 3 for description.)

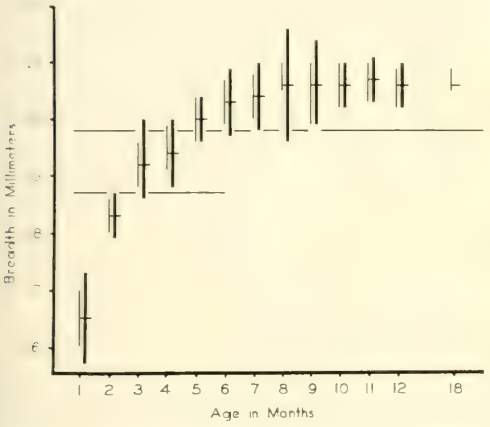

Figure 11. Zygomatic breadth of known-age cotton rats. The thinner, vertical line on the left of each symbol represents the range of the data. The heavier, vertical line on the right of each pair represents two standard deviations on either side of the mean, which is indicated by the horizontal line.

separated by sex especially considering the small sample size.

The measurement of the most apparent value for age determination is the zygomatic breadth (Fig. 11). For this measurement the standard deviation was computed and added to the figure as two standard deviations on either side of the mean. This procedure was used to determine the validity of the sample as the two standard deviations would thus represent ninety-five per cent of the population and provide more comparison than the actual ranges.

By inspection one-month-old individuals are distinct from all other groups. The two-month-old individuals are nearly distinct. A line has been added to the figure at 18.7 mm to separate the two-month-old individuals from older specimens. The overlap of the data is in the standard deviation only, the ranges being distinct. Three- and four-month animals together form a group which is largely separable from the rest of the animals in this study. A second line has been added at 19.8 mm which, although arbitrary, separates most of the three- and four-month specimens from most older individuals.

Thus, four age groups can be identified: 1) one-month-old individuals; 2) two-month-old individuals; 3) three- and four-month-old individuals; and 4) individuals five months or older. These categories have virtually no overlapping of the ranges. The sample size is undesirably small, perhaps accounting for a large standard deviation.

Alexander (1960) has shown that there is approximately a 1.5% decrease in the zygomatic measurement of the muskrat as the skull dries. This shrinkage in cotton rats would amount to 0.3 mm of a zygomatic breadth of 20 mm. Since all skulls were cleaned and measured at the same time, the shrinkage should be uniform and the data still valid. Presumably standard cleaning and measuring techniques should be used when making this measurement on a population sample. Character indices also were computed by various combinations of the four measurements, but the applicability of the data was not increased.

Older animals exhibit a general increase in the development of the ridges or crests of the skull. The ridges appear to attain most of their development by three or four months, although the skull still appears to become more massive throughout the age groups. However, with the exception of the breadth of the skull at the lambdoidal ridge, all measurements to evaluate this increased size were unsuccessful. Weighing the skulls only served to distinguish one-month-old individuals as a group. Furthermore, when guessing the age of an individual skull by examination of the development of its ridges and its weight, I was successful only with one- and two-month-old individuals. Thus, while subjectively evident, the ridges defy objective measurements and are concluded to be of little value for age determination.

The mandibular weight permitted the identification of both one- and two-month-old animals as groups distinct from the rest of the specimens. The limited value of this measurement does not justify the time spent in the careful cleaning of the mandible before weighing.

The forelimb was studied extensively for areas of epiphyseal fusion that would be of value for age determination. The results described below are a composite of both the maceration and X-ray data, the former being the more easily studied. At one month the distal epiphyses of the metacarpals are distinguished from the diaphyses by a nonstaining band of cartilage, which by two months is reduced to a deeper staining suture line. This line becomes indistinct at three months and is absent at four months of age.

The sesamoid bones of the metacarpal-phalanx joint first become apparent by staining at three months of age, but are distinct from the two bones of the digit. At four months the sesamoids are beginning to fuse with the metacarpals, which process is completed at five months of age.

A second series of sesamoids can be seen at four months, at the distal end of the proximal phalanx. Like the first series these bones are fused to the proximal phalanx two months later at an age of six months.

The distal epiphyses of the radius and ulna at one month are distinct and separated from the diaphyses by a clear nonstaining band of cartilage. For animals two months of age the epiphyses are separated only by a line lacking in stain. The epiphyseal suture is about one-half ossified at three months. The process is virtually completed at four months although a suture line is visible in all older specimens and shows very little change. Green (1949) also has noted the persistence of this suture line to older ages in the Norway rat.

The proximal epiphysis of the ulna is very similar to the distal epiphyses of the radius and ulna, clearly distinct at one month and largely ossified to the diaphysis at four months of age. A suture line is similarly present in all older specimens.

The acromion process of the scapula shows no ossification at one month of age and only a small distal area of stain deposition at two months. The amount of ossification gradually increases so that the process is largely if not completely ossified at five months. A suture line is still visible for a few months, but is absent in all specimens nine months or older.

The suprascapular cartilage is also unstained at one month of age. At two months a small area of staining appears at the corner of the glenoid and vertebral borders of the scapula. By three months the suprascapular cartilage is ossified about one half its length, but a narrow stain-free area separates the ossifying cartilage from the scapula. At five months of age the cartilage is largely ossified, and only at this time does the suprascapular cartilage begin to fuse to the scapula. This later process continues slowly and is not complete even in the twelve-month animals.

The ossification processes are not all complete at six months, but at this age the changes are very slow and irregular. These later stages of ossification and fusion are of very limited value for age determination and are not discussed. All the described areas of ossification are summarized in Figure 8. Other epiphyseal areas exist in the forelimb, but these are either difficult to distinguish, such as the epiphyseal areas of the humerus, or proceed too rapidly to be diagnostic, such as is seen in the epiphyses of the phalanges.

The tail also was X-rayed to study the degree of ossification and fusion of the intervertebral discs. This procedure was investigated as the tail can be X-rayed easily on a live, anesthetized animal where an examination without injury is highly desirable.

For the younger animals the caudal intervertebral discs are largely unossified to the centra of the vertebrae. Those that are nearest the pelvis ossify first. The degree of ossification increases with increasing age of the specimen, although it is never complete. Even in the twelve-month-old specimens the last few (three to six) discs remain quite distinct. The major difficulty in evaluating the data is deciding which discs have begun to show ossification in order to produce numerical or even reasonably objective data. No satisfactory method was found. The problem was compounded due to the X-ray film used and its inability to produce a sufficiently sharp outline of the structure.

The use of epiphyseal fusion gives promise as an aging device. While the best data are derived from the examination of macerated material, the use of X-rays is encouraging

and should be investigated further. If macerated limbs are used, a good estimation of age can be made by the examination of the seven areas of bone development (Fig. 12).

F. Lens Weight

All lens weights discussed are the combined weight of both lenses. The technique employed here is that described by Lord (1959). Each age group was examined for sexual dimorphism. Except for the ten-month-old group discussed below, no significant differences exist between the sexes ($P>0.3$). The data for both sexes are therefore considered as a single sample for each age, the larger sample size permitting a better mathematical comparison of the age groups. Lord likewise noted no differences between males and females and considered both sexes as a single sample.

The ranges and means for the data are presented in Figure 13. The standard deviation has been computed for each age group and added to the figure as two standard deviations on either side of the mean. This method of evaluation is especially justified since the standard deviations exceed the range of most samples.

The one-month-old sample is quite distinct. The two-month-old sample is nearly so, a small overlap of the standard deviations occurring between the two- and three-month samples. Animals up to three months, therefore, could be quite correctly aged by this technique. For all older age groups, both the range of the sample and the area covered by the two standard deviations show at least some contiguity, especially for those groups six months or older.

Older age groups might be distinguished by making arbitrary weight values to separate them. Accordingly, horizontal lines have been added to Figure 13 at 25 and 33 mg. This permits isolation of a four- and a five-month-old age group from the three-month age group and the age group six months or older. Thus a total of five age classes can be established. Of the 62 individuals in the one- through six-month-old age groups, only three, or less than 5%, thus fall into incorrect classes. Therefore, the dry lens weight would appear to be a very satisfactory technique for age determination.

For the successful application of any aging technique, one should be aware of possible variations and their causes. The 10-, 11-,

<table>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th></tr>
<tr><td>D. Epiphysis, Metacarpal</td><td>Separate</td><td>Fusing</td><td colspan="4">Fused to Metacarpal</td></tr>
<tr><td>Sesamoid, Metacarpal</td><td colspan="2">Nonstaining</td><td>Stained</td><td>Fusing</td><td colspan="2">Fused</td></tr>
<tr><td>Sesamoid, P. Phalanx</td><td colspan="3">Nonstaining</td><td>Stained</td><td>Fusing</td><td>Fused</td></tr>
<tr><td>D. Epiphysis, Radius, Ulna</td><td rowspan="2">Separate</td><td colspan="2" rowspan="2">Fusing</td><td rowspan="2">Fused</td><td colspan="2" rowspan="2">Suture Line Visible</td></tr>
<tr><td>P. Epiphysis, Ulna</td></tr>
<tr><td>Acromion Process</td><td>Nonstaining</td><td>$\frac{1}{8}$ Stained</td><td>$\frac{1}{3}$ Stained</td><td>$\frac{2}{3}$ Stained</td><td colspan="2">Suture Line Visible</td></tr>
<tr><td>Suprascapular Cartilage</td><td>Nonstaining</td><td>$\frac{1}{4}$ Stained</td><td>$\frac{1}{2}$ Stained</td><td>$\frac{3}{4}$ Stained</td><td colspan="2">Fusing to Scapula</td></tr>
</table>

Figure 12. Summary of data on epiphyseal fusion. The numbers at the top indicate the age in months.

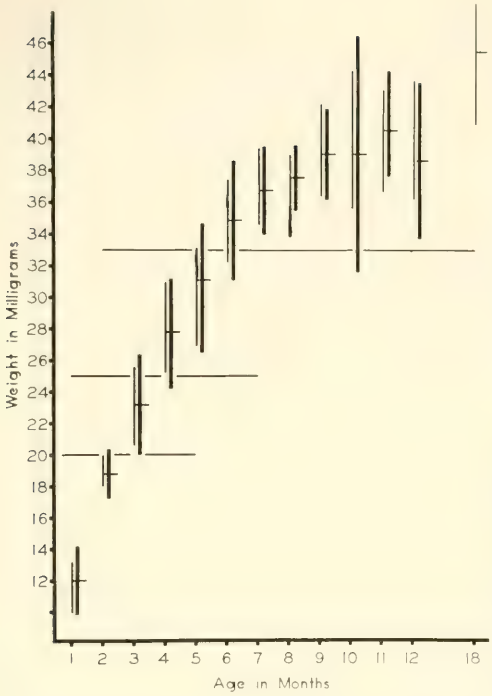

Figure 13. Dry lens weight of known-age cotton rats. (See Figure 11 for description.)

and 12-month-old samples offer some insights to the problem. The limits of the two standard deviations of the 10-month sample considerably exceeded the observed range, admittedly large itself. The individuals constituting this sample fall into two groups: one of six females whose lens weights range from 35.6-37.8 mg, and a second group of three males whose lens weights ranged from 43.9-44.3 mg. This is the only age group where distinct differences occur between the sexes. Perhaps a more reasonable explanation is that the females were not autopsied until several days after being sacrificed, although the specimens were frozen. Montgomery (1963) has shown that raccoon lenses lose weight after being frozen for several days before autopsy.

For the 11-month-old sample, one individual born in May and sacrificed the following April had a lens weight of 36.7 mg. The remaining individuals of this sample were born in September and sacrificed the following August. These individuals had lens weights ranging from 40.4 to 43.2 mg. The 12-month-old samples contained one group of seven individuals born and sacrificed in May whose lens weights ranged from 36.2 to 39.4 mg. The remaining four individuals of the sample were born and sacrificed in September and had lens weights of 39.6 to 43.8 mg. None of the specimens were frozen prior to autopsy. An explanation of the differences in weight might be variation induced by the day length since the animals, born in different seasons, were subjected to the normal day length throughout this study. Also, since the animals were from several different litters this range of weight might simply indicate normal variation in the older animals.

G. Field Study

The last phase of this study was an attempt to obtain accurate growth data from the natural habitat to be compared to the laboratory-derived growth curves which could then be evaluated and adjusted. An old field habitat was selected for releasing four-week-old laboratory-raised animals. The area had been trapped intermittently over a period of two years and was known to be suitable for cotton rats. For two months before releasing the young animals I made several trips to the area to trap out as much of the existing small mammal fauna as possible, reducing competition for the young cotton rats. During the retrapping period following release of the young, only one nonmarked cotton rat was collected.

Each cotton rat to be released was toe-clipped for identification when weaned. Three or four cotton rats from the same litter were then placed in a new cage. In place of the usual tin-can nesting facilities the animals were provided with a wooden nest box approximately 6 x 6 x 4 inches high. A 2 x 2 inch opening was provided with a cover that could be closed quickly with the cotton rats inside. The nest box was transported to the release area, the cover opened, and the animals left with an available nest to which they might be accustomed. A liberal supply of food was also placed in the nest box. I had hoped that some individuals would continue to use this shelter and thus facilitate their recapture. Many of the nest boxes appeared to be inhabited as grass and cuttings were incorporated into the nest box cotton. However, only one cotton rat was ever found in the nest box and this individual escaped before the opening could be covered. A total of 96 rats were released from March 25 to June 5, 1961.

During May the release area was exhaustively retrapped on three occasions with collapsible Sherman live traps. Approximately 200 traps were set each of the seven nights involved. The release area was also live-trapped in mid-June for five nights continuously with 240 traps per night, prior to which heavy rains had inundated the release area. No cotton rats or any other small mammals were collected during the latter period. The field project was terminated at this time for lack of additional young available for release.

Cotton rats trapped in May were etherized, weighed, and measured, and examined for molting and reproductive status. They were then allowed to recover for 30 minutes and released at the same location where they were trapped. Of the 96 rats released, 12 were recaptured from one to four times for a total of 23 recaptures. Four individuals died in the live traps, presumably from the high temperature due to direct sunlight on the traps and from attacks by fire ants. The remains of the four that died were autopsied as far as possible. The date on these four animals are given in Table 2.

The weights and body lengths of all retrapped cotton rats are plotted in Figures 14 and 15. If an individual was retrapped on several consecutive days, only the first day is plotted. Two individuals were recaptured a second time, 22 days after their first recapture. These points are connected with a line for identification.

As expected, weight increase was much slower in the field than in the laboratory. The lines on Figure 14 represent the *minimum* weight of the monthly laboratory sample of each sex. The mean weight was so much greater that plotting it on the chart would have had no value for comparison.

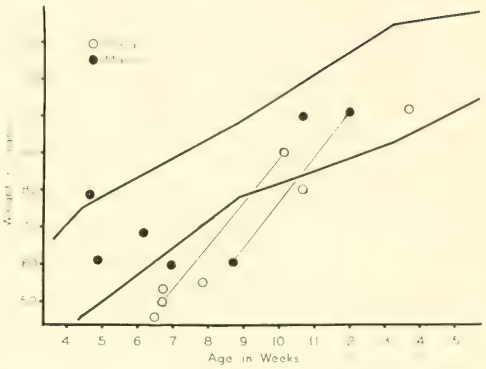

Figure 14. Body weights of released known-age cotton rats. The heavy line represents the *minimum* monthly weights from Figure 1 (upper line for males; lower line for females). The thin lines connect data for the same individual.

The difference between the laboratory and field studies probably is due to increased activity in search for food—food that also is less likely than the laboratory diet to be accumulated as body fat. Possibly the stress induced by being released into an unfamiliar and competitive habitat and/or being in the live-trap for extended periods may have affected the body weight. Animals recaptured on successive days showed weight losses from previous days of up to five grams. The information on body weights is too limited to construct a growth curve of wild animals. The data do strongly suggest that laboratory weights are of little use for age determination of wild individuals.

The body length measurements are plotted in Figure 15. The lines in this figure represent the *average* body length of each monthly sample. The field data appear to be quite consistent with the laboratory data, and substantiate the idea that the greater body

TABLE 2.
Data on released animals that died in the live traps. Skull measurements in millimeters; lens weights in milligrams.

Animal No.	356	382	426	445
Sex	m	f	f	f
Age (days)	85	75	45	55
Condylobasilar length	34.0	32.6	30.5	32.4
Nasal length	12.7	12.1	11.1	12.5
Lambdoidal breadth	13.6	13.3	13.0	13.2
Zygomatic breadth	18.8	18.4	17.6	18.0
Lens weight	22.8	*	13.4	*

* Data not available

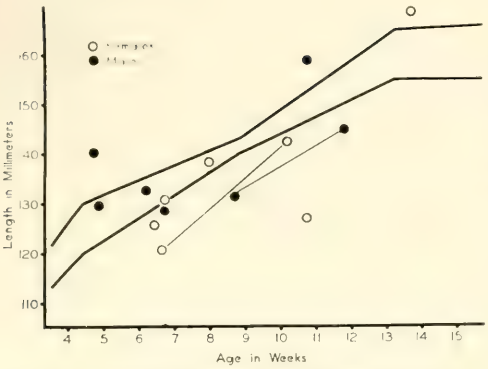

Figure 15. Body length of released, known-age cotton rats. The heavy lines represent the *mean* body lengths from Figure 2 (upper line for males; lower line for females). The thin lines connect data for the same individual.

weights of laboratory animals reflect a greater amount of fat and not larger size. On the basis of these limited data, body length appears to be a useful method for age determination.

Five cotton rats were found to be molting when recaptured. Two individuals (33 and 34 days old) were completing the subadult molt. Two others (61 and 75 days old) were in the earlier stages of the adult molt. A single recapture at 84 days of age was molting dorsally, thus completing the adult molt. Five individuals captured at 43-47 days of age were not molting. Presumably they were in complete subadult pelage and would shortly begin to molt to the adult pelage. The cotton rat examined at 96 days was not molting. The individual molting data of the released animals coincide well with the observed laboratory data except that the subadult-to-adult molt may begin somewhat later and end somewhat earlier. With more field studies and an increased understanding of seasonal variation, the molting pattern for age determination could possibly be developed to a high degree.

The reproductive data are limited. One male died in the trap (age 84 days). A smear of the testis and epididymis was negative but this is attributed to the carcass being severely damaged by fire ants and the length of time between death and microscopic examination. All males when recaptured had the testes in the scrotal position.

One female captured at 96 days of age had considerably enlarged teats and was lactating. The vaginal orifice was imperforate which suggests that parturition had occurred one or two days previous to capture. With a normal gestation period this female probably mated at about 65 days of age. This single example of reproductive activity of a released female agrees well with the laboratory data. The reproductive tracts of the three females that died in the live traps did not show any indication of reproductive activity.

The teeth of the four released cotton rats that died in the traps showed the most dramatic difference from the laboratory study. Staining of the teeth, presumably due to the diet, is very marked. The teeth are considerably more worn than laboratory individuals of the same age. Using laboratory tooth wear as a criterion for age determination produces an estimate two-to-four months older than the actual age. Since tooth wear is more rapid in the natural habitat, this character might be used successfully for aging, as more variability would be present and thus more stages of wear that possibly would reflect the animal's age.

The skeletal characteristics of the released cotton rats compare well with the laboratory data. The skull measurements are listed in Table 2. The condylobasilar and nasal lengths both fall in the lower range of the known-age measurements. The lambdoidal breadth and the zygomatic width show very satisfactory agreement with the laboratory data. A good age estimation can be obtained on the basis of these two measurements..

The characteristics of the stained forelimb are in consistent agreement with the laboratory data. The metacarpal and phalangeal epiphyses, the distal epihyses of the radius and ulna, the proximal epiphyses of the ulna, and the ossification of the acromion process and the suprascapular cartilage are all in close agreement with the laboratory data. These limbs were examined initially without the knowledge of the specimen's exact age. The 45-, 55-, and 75-day-old specimens were all estimated to be "about two months" while the 85-day-old individual was estimated to be "three months." These estimations appear to be as accurate an estimation as could be made with any character.

The lens weights of only two of the four dead known-age individuals were in satisfactory condition for study. The eyes of the

others were dried out and partially eaten by ants. As can be seen by comparing the lens weight from Table 2 with those in Figure 13, the data from the released cotton rats fit the laboratory data quite satisfactorily.

V. Discussion and Conclusions

When considering the reliability of age determination based upon laboratory data, two points must be kept in mind. First, animals are not found in month-old groups but form a continuum of all ages. Thus, while one may attempt to assign an animal to a certain age group one does so with the knowledge that the age of the animal is being approximated only. The second point is estimating age from a live or a dead animal. In a live-trap study one must determine the age and then release the animals unharmed. Such a procedure limits the number of characters that can be employed. The present study was based largely upon the interpretation of characters from dead animals. I presumed that some dead-animal characteristics could be adapted to evaluate living cotton rats. One possible adaptation would be the use of X-raying in place of the maceration and staining technique.

From an examination of the data that have been presented it appears that age determination can be accomplished best for animals up to six months of age by a combination of several characters. Age determination of older cotton rats appear virtually impossible other than to indicate an individual as being over six months of age. Precisely what age cotton rats may attain in their natural habitat is, of course, unknown. Some information on this topic has been presented by Odum (1955), who stated that an animal once trapped was never retrapped more than six months later. Assuming such an individual to be a month old or more when initially trapped, its maximum age when retrapped would probably not exceed seven to ten months. In a live-trapping study of the cotton rat in Louisiana in which I participated, cotton rats were never retrapped more than four months after the initial trapping. Recently Dunaway and Kaye (1964) did note two wild cotton rats approximately 10 and 11 months of age. It is not known how many other rats attained such an age.

The techniques developed in this study are felt to be satisfactory for estimating the age of wild cotton rats throughout the greater portion of their presumed life expectancy. However, any attempt to estimate age should be based on several characters to minimize error. The most reliable characters are summarized in the following two paragraphs.

The computed body-length measurement is rapidly collected and is satisfactory to separate animals up to three months of age from all other animals of the population. Molting might also be used to distinguish the younger age categories, although the distinctions are less specific than body length, and the process is more easily studied from the skin side of the pelt. The molting pattern continues to change fairly regularly in the laboratory specimens, but the information is probably inadequate for use as a critical age-determining characteristic, as molting has been shown in other rodents to vary with the seasons of the year. This particular character definitely should be studied in more detail under more varied laboratory conditions and in the natural habitat.

Epiphyseal ossification can be determined on a dead animal by maceration and staining. By comparing the several ossification areas, one can establish the approximate age of an animal to six months of age. For a live animal, epiphyseal ossification might be studied by X-raying the limb of an anesthetized animal. This character may have considerable advantage as an age-estimating technique, as ossification processes seem to proceed at a fairly constant rate and presumably are not as subject to environmental variation as are body weight and molting. One additional charatcer that might be developed for determining the age of a live cotton rat is the measurement of the zygomatic breadth. This character was studied only on clean skulls but proved quite accurate for estimating age. Since the amount of flesh covering the zygomatic arch of the live animal is quite limited, this measurement might be adapted for estimating age. The lens weight data are very useful for age determination. There is no clear separation of the four- and five-month-old animals, but these two ages are distinguishable from all younger age groups and from all older animals. As the moment nothing is known of the factors, other than freezing, that may cause variation in the lens weight. Thus by a combination of the above techniques, one should be able to closely approximate the

age of a given animal based upon the laboratory data.

One very difficult problem to evaluate is the difference between growth of a laboratory specimen and growth of a wild specimen in its natural habitat. That such a difference exists is easily shown by the fact that the laboratory cotton rats used in this study were considerably heavier than their counterparts released in the field at an age of 4 weeks. However, this may be only part of the picture. Unknown and impossible to evaluate is the effect of preweaning growth on the cotton rat before it is released. All cotton rats, prior to release, received what might be considered an adequate if not optimal diet. Presumably by this time the subsequent growth pattern has been largely determined. Similarly difficult is the problem of evaluating the health and nutrition of the female cotton rat prior to conception. A female in good health could be expected to produce healthier young than a female in poor health. Thus not only the early preweaning growth but even the prenatal development of the animals that were released was undoubtedly affected by the laboratory environment. One possible mechanism for evaluating such changes would be to collect pregnant cotton rats from the field and allow them to have their litters in the laboratory. Immediately after birth the animals could be toe-clipped, and then the female and her litter released into the natural environment, possibly in a large enclosure. Dunaway and Kaye (1964) toe-clipped young cotton rats born in live traps. Presumably these individuals are the known-age animals they discuss. This method is the best way of obtaining known-age animals in their habitat, although perhaps limited in numbers of individuals.

Several studies have attempted to estimate the age of the cotton rat. Erickson (1949) classified each individual as either immature or adult but did not give any basis for these age classes. Since the basic purposes of the study were calculations of movement and density, age determination was not particularly important.

Similarly, Stickel and Stickel (1949) studied the home range of the cotton rat in Texas. They recognized four age classes based primarily on size and breeding condition. Only approximate body-length measurements were taken, and these were not listed by the authors. The age classes were thus rather subjectively determined. Their two youngest age classes probably correspond to one- and two-month-old animals as used in this study.

Sealander and Walker (1955) conducted a study of the cotton rat in northeastern Arkansas. Age classes were initially defined on the basis of actual or potential reproduction. Thirty days was considered the age at which cotton rats might begin breeding. The laboratory growth data of Meyer and Meyer (1944) were then employed to form weight limits to each age class. The age classes were subadult, 10 to 29 days; young adult, 30 to 50 days; old adult, 51 to 250 days. On the basis of the age classes as determined by using body weight, these authors found an age distribution during the late winter and early spring that indicated a high percentage of young individuals in the population. This, in spite of the fact that breeding had ended the previous November and had not yet resumed. Thus weight used as an age criterion did not produce an age distribution that agreed with the field data. These authors also noted that considerable body fat accumulated by the cotton rats in November and December, declined drastically in January and February to about 60% of the peak December value, and disappeared in April. Presumably this body fat is used as an energy source during the period of its decline. Thus, while the animals are actually becoming older they are losing weight, a fact which would place animals in younger age categories. In theory, weight might be used as an aging technique during the severe winter period if one could take into consideration the probable weight loss in each individual at this time. However, it would be incorrect to assume that the same rate of body weight decline is present during each winter.

Odum (1955) concurrently studying cotton rat populations over a period of 11 years, presents data that may give a truer picture of the normal weight gain. He captured a 15-gm female on June 6, 1949. On the basis of an average birth weight of 7 gm and a gain of 1 gm a day (Svihla, 1929; Meyer and Meyer, 1944), the above individual was adjudged to be a week old when captured. It was recaptured on August 21, at a presumed age of 159 days and a weight of 96 gm. A second female weighed 74.5 gm on

August 21, and two and one-half months later, on November 9, weighed 103 gm. Judging from my release records, this second female was two to three months old when first caught and, therefore, about five months old at the weight of 103 gm. While limited, the data do give some idea of the rate of weight gain by wild cotton rats. According to the weight limits of the age categories employed by Sealander and Walker, these two females would have been classified as young adults (age 30-50 days).

Odum's study of cotton rat populations was based largely on spring (May) and fall (November) trapping. The primary purpose of his study was to follow the periodic changes in population density. The trapping program employed served the purpose well, as Odum's methods avoided the problematic period of winter weight loss.

Dunaway and Kaye (1964) mention a male and two female cotton rats weighing 103, 95, and 101 gm, respectively, at an age of 104 days when trapped in November. A male and a female 119 days of age when captured in February weighed only 88 and 77 gm, respectively, however, and a 117 day old male weighed only 75 gm at this time. Also, a female, age unknown, weighed 62 gm in September, 87 gm in November, but only 84 gm in February. In addition to emphasizing the severe effects of winter, the data point out the impossibility of using laboratory weight for age estimation. The three 3½-month-old cotton rats trapped in November fall in the weight range of 2 month old laboratory rats. Using Odum's (1955) criteria, these animals would have been placed in the 2-to-5-month-old category.

The information on growth rates and reproductive activity of known-age individuals in the natural habitat is too limited for specific conclusions. However, where available, data from cotton rats released in the present study either show close agreement with the laboratory data or else give indication that the laboratory data might be modified to allow for an adequate age estimation. Body weight is a useful age-determining technique at certain seasons of the year, but should not be employed during the period January-through-April except with extreme caution. The other techniques described in this study should be more useful than weight for age determination, especially during the winter. Finite evaluation of age-determining techniques should be based on releasing and retrapping young known-age individuals at all seasons of the year.

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