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Reproductive Correlates of a Perineal Gland in the Hispid Cotton Rat

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ABSTRACT

During studies of the annual cycle of reproduction in the hispid cotton rat (*Sigmodon hispidus*) in southeastern Virginia, we discovered an anal (more specifically, perineal) gland that is present only in males during the breeding season. The perineal gland encircles the lower end of the rectum and has ductal connections to the urethra, through which its secretions probably are delivered. This fatty gland is highly developed in breeding males but, like the testes and seminal vesicles, regresses during the winter non-breeding season. The prominence and cyclicity of the perineal gland suggests that it somehow facilitates normal reproduction. The combined mass of testes, seminal vesicles, and perineal gland constitutes only about 0.01 percent of the body mass of large males during the non-breeding season, but as much as 4.8 percent of body mass during the breeding season. Thus, males devote a large amount of energy to growing and maintaining these glands in anticipation of and during the breeding season. Despite two field trials, the function of the perineal gland and the nature of its secretion are unknown.

INTRODUCTION

The hispid cotton rat, *Sigmodon hispidus*, the biology of which was reviewed by Cameron and McClure (1988), is a native rodent of old fields and early successional habitats in the southeastern US. Seasonal breeders everywhere, in southeastern Virginia cotton rats breed from March to October (Rose and Mitchell, 1990; Bergstrom and Rose, 2004). The onset of male reproductive capacity, using the criterion of convoluted cauda epididymides (Jameson, 1950), extends beyond the interval of female reproductive readiness by 2-4 weeks on both ends. During studies of reproduction in *S. hispidus*, one of us (RKR) noted, in adult males during the breeding season, the presence of a large subcutaneous anal organ, hereafter called the perineal gland because it encircles the rectum near the base of the tail. Although large in breeding males, this organ is indiscernible in non-breeding males, in females, and in juveniles.

In order to evaluate its nature, we examined the gross anatomy and seasonal changes of the perineal gland throughout the annual cycle. The objectives of our study were to: (1) describe the anatomy and seasonal cyclicity of the gland, (2) determine the relationship between the gland and the reproductive system, and (3) if possible, determine the method of delivery and information content of the glandular product(s). To accomplish these goals, we recorded changes in the statuses of the perineal gland, testes, and seminal vesicles by the necropsy of monthly samples of adult-sized males. We learned that the perineal gland is a seasonally cyclical multilocular gland, by inference is under androgen control, and probably delivers its secretions via the urethra.

MATERIALS AND METHODS

Specimens of cotton rats for necropsy were obtained using Fitch live traps (Rose, 1994), baited with Purina Sweetena®, a mixture of oats, corn, millet, and molasses, placed about 5 m apart on transect lines in old field habitat. Monthly samples of potentially adult males (> 50 g) were obtained from four old fields in southeastern Virginia. After chloroform euthanasia, standard body measurements including lengths of tail, hind foot, ear, and body (total length minus tail length) and weights of body, paired testes, seminal vesicles, and perineal gland were determined using a Mettler analytical balance. In all, 129 males were necropsied, 90 from the breeding season and 39 from the non-breeding season.

To prepare tissues for histological examination, a subset of wild-caught animals was anesthetized with chloroform in the lab, after which an intraperitoneal injection of 1.0 ml of tribromo-ethanol (1 g/40 ml) was used to maintain anesthesia during perfusion. The thoracic cavity was opened, a cannula was placed in the left ventricle for introduction of perfusate, and the inferior vena cava was cut to allow the exit of fluids. The tissues were flushed with heparinized buffered saline and then perfused with a solution of 4 percent paraformaldehyde in 0.1 M phosphate buffer solution (Glaubert, 1975). After the cotton rat had succumbed, the perineal region was dissected away from the lower torso and stored in 10 percent formaldehyde fixative for 24 h. Then, after the pelvic girdle and lower spine were carefully removed, the block of tissue was placed in 10 percent formalin until prepared for embedding about 20 h later.

Fully developed perineal glands could not be sectioned successfully because their waxy contents prevented adequate fixation, causing the paraffin sections to shatter. The most readable slides were prepared from a 75-g male with scrotal testes and a partially enlarged perineal gland that allowed dehydration and fixation. After the bones had been removed, the remnant was divided into three smaller pieces before dehydration. The caudal piece included the majority of the perineal gland, the central piece included a large portion of the penile shaft, and the most craniad piece, which extended well into the pelvic cavity, included the external penis, seminal vescicles, and preputial glands. These pieces were placed for dehydration in successive 30-minute baths of 25, 50, 70, and 80% ethanol before being embedded overnight in paraffin in an Autotechnicon-2A automated tissue processor. The paraffin blocks were thin-sectioned with an American Optical 820

microtome. The 10μ sections were stained with either a modified Periodic Acid Schiffs (PAS) or a Mayer's hematoxylin and eosin (H & E) stain (Luma, 1968).

The first field study, using the homogenate from whole perineal glands placed on filter paper and inserted into experimental traps, was conducted on a 6 by 5 grid with each trap being placed 7.5 m apart. Before the experiment, the plot was trapped, and animals ear-tagged and released, for 19 days in September and early October to determine a population estimate (MNA), sex ratios, and reproductive status. The perineal homogenate was produced using the frozen perineal glands of necropsied mature males; 12.3 g of glands from several males was homogenized in 45 ml of a 70% ethanol:30% phosphate-buffered saline (PBS) solution which contained 200 mg/l of L-ascorbic acid. (The ethanol was used as an organic solvent to solubilize any lipids, the PBS was used to stabilize the pH, and the L-ascorbic acid inhibited oxidation. This three-ingredient solution on filter paper triangles was used as the control for the experiment.) Both homogenate and control solutions were then stored at -20 ºC until used in the field studies. Later these mixtures were used to saturate 4 cm by 5 cm triangles of Watman #11541 filter paper, which were then placed inside traps; traps were checked daily and papers were replaced every third day. The field experiment was run for 10 days near the end of the breeding season, during which captured animals were evaluated for their reproductive status and body weight.

The second field study, conducted near the end of the breeding season the next year, examined the effect of chemically separated fractions of the perineal gland homogenate on wild populations of cotton rats. Perineal gland and muscle tissue from the flanks of male cotton rats (as control) were homogenized separately in PBS (1g/4ml). These homogenates were extracted three times with a 9:1 chloroform:methanol solution; the methanol/water fraction was kept at -20 ºC until use, whereas the chloroform fraction was evaporated off under a gentle nitrogen stream and then reconstituted to a volume of methanol equal to the volume of the top fraction. For the field study, a 15 by 6 grid (7.5-m interval) was established and animals were trapped, marked, and evaluated as before. Fifteen traps were assigned to each of the six treatments in a stratified random fashion. Each trap was baited with a 0.1 g sample of either perineal gland or muscle homogenate, or a chloroform or methanol extract (extracted from 0.1 g of homogenized tissue). These samples, on #10 Watman filter paper triangles, were placed in traps as before. The traps were checked daily for 10 days; fresh papers were added after the fifth night.

The SAS statistical package (SAS Institute, Inc., 1985) was used for standard statistics, general linear model analysis of variance (ANOVA), stepwise regression, and correlation analysis. Unless otherwise stated, the $P < 0.05$ level of significance was used.

RESULTS

During the breeding season, the adult male cotton rat possesses a large perineal gland (Fig. 1) located dorsal to the testes, under the tail, and just anterior to the anus. The testes must be excised in order to see the position of the gland in relation to the rectum, penis, and gracilis muscles of the hind legs. The anterior-most aspects of the gland

originate at the base of the penis, and when fully developed, the gland sometimes extends dorsoposteriorly to the fourth caudal vertebra.

The perineal gland is divided on each side into three unequal parts, which together encircle and are loosely attached by fascia to the rectum (Fig. 1, numbers 3A, 3B, 3C). The segment located anterior and ventral to the rectum is large (Fig. 1, 3C) and has strong, definite attachments to the base of the penis. The other smaller lobe-shaped segments (Fig. 1, 3A, 3B) lie dorsal to the rectum and caudad to the attachment points of the largest segment. One region, distinctly separable from the largest mass of the gland (Fig. 1, 3A), has a lobular appearance and dorsally flanks each side of the rectum. It is held to the largest glandular mass by a single fibrous tubule (observed but not characterized histologically) and loosely by fascia elsewhere. Due to its placement and description, these 3A lobes may not be part of the perineal gland but rather Cowper's glands, as described in the porcupine (Mirand and Shadle, 1953). (Cowper's glands are accessory to the testes and produce the vehicle to transport sperm.)

The size, weight, and shape of the perineal gland, which literally rings the rectum (Fig. 2), can vary dramatically depending on breeding status, which is determined by the maturity of the animal as well as the season. When a large (> 80 g) male is in the non-breeding condition, the gland is greatly reduced and often difficult to detect. As the breeding season approaches, the gland enlarges, revealing three definite bisymmetrical regions on each side (Fig. 1), as described above.

Histology of the perineal gland

The excised perineal gland of a large male in breeding condition is approximately the size and shape of an asymmetrical Lifesaver®. The gland is fatty and waxy, and resistant to penetration by the fixing and dehydrating agents used to prepare histological tissues for staining, and thus sections cut from such tissues were not revealing. After much trial and error, we succeeded in making tissue sections from the growing glands of young males but not from those of fully mature cotton rats.

The low magnification photograph of the cross-section of the pelvic region (Fig. 3) shows the rectum (#1), two lateral (perineal) glandular regions (2A, 2B) with their muscular investments lying lateral to the rectum and below the urethra (#3), and two other areas of glandular tissues also lateral to the urethra (#4). The glandular tissue shows bilateral symmetry, with portions lying either lateral or dorso-lateral to the rectum and ventral to the urethra. The glandular areas contain small tubules that coalesce to form larger tubules or ducts, which in turn lie within a neck-like projection of the gland, possibly connecting with the urethra. Two additional structures, which flank the urethra, contain ductal fibrous connective tissue with squamous epithelium and some vasculature. These appear to provide structural support for the perineal gland. The vicinity of the gland is highly vascularized, with large extravascular and sinusoidal spaces. Although the perineal gland and the rectum are closely apposed, no connections were detected.

Higher (31X) magnification (Fig. 4) revealed the tri-partite gland surrounded by a prominent investment of smooth muscle. The bulbous portion of the gland contains

secretory acinar cells, connective tissue, and small tubules. The neck-like projection of the gland has narrowing ducts extending towards the penis, all surrounded by smooth muscle. H & E staining revealed tubules filled with a pink mucinous material similar in appearance to colloidal tissue of the thyroid gland. The material is further characterized using a PAS hematoxylin stain, creating a bright pink similar to stained glycoproteins or glycolipids.

At high power (not shown), the tissue is seen as a multi-lobular, compound tubuloacinar gland with several distinct bundles separated by septa and connective tissues. Individual lobules are surrounded by fibrous connective tissue and the entire group of lobules is enclosed by smooth muscle. Tubules within and between lobules are lined with epithelium progressing from columnar to more cuboidal in shape. The non-tubular regions reveal the mucin-filled acini, resembling alveoli. Each acinar (secretory) cell has a large granular cytoplasm and a flattened nucleus located near the basal lamina. Overall, the acinar cells are similar in appearance to the acini of salivary gland tissue.

At the head-like junction of the perineal gland with the penis (not shown), the gland contains reduced numbers of acinar cells with more connective tissue and glandular tissue such as that found in the more lobular portions of the gland. However, as this head abuts the penis, the nature of the glandular tissue within the muscular wall of the penis is different from tissue outside of the muscular wall, consisting of densely staining columnar tissue with larger nuclei and little cytoplasm when compared to the glandular tissue outside of the muscle. These glandular areas are also surrounded by blood-filled sinusoids. This type of tissue extends cranially up the dorsal portion of the penis and can also be seen in low-magnification cross sections from the second, more craniad block of tissue in the same plane as the vas deferens (photograph not shown).

Seasonality of the perineal gland

In order to illustrate the seasonal variation and relative importance of the perineal gland, standard statistics were determined separately for the entire sample and for the non-breeding and breeding seasons (Table 1). The results showed the relative uniformity in both lengths of body measurements and body weights among the three groups, but drastic and significant differences in the weights of testes ($F = 101.34$, $P < 0.001$), seminal vesicles ($F = 45.48$, $P < 0.01$), and perineal glands ($F = 45.65$, $P < 0.01$) between non-breeding and breeding seasons. The striking seasonal change in the weight of the perineal gland illustrates its potential importance. Even with the inclusion of young (short and light) males, the mean weight of the perineal gland in the breeding season (813 ± 65.2 mg SE) comprised about 0.8 percent of the grand mean weight for the 90 males. The perineal gland weighed as much as 1.4 percent of total body weight in the largest males.

To further examine the seasonal variability of the perineal gland, a stepwise regression analysis was run using perineal gland weight as the dependent variable. Of the six independent variables (weights of body, testes, and seminal vesicles, and lengths of body, hind foot, and ear), only the three weights met the significance level for entry into the model. However, body weight was dropped from the model after it was determined

to be 'not significant' when only the three organ weights were entered into the model. Thus, this analysis confirmed that the perineal gland and the reproductive organs (testes and seminal vesicles) show seasonal changes, whereas body weight and the linear measurements do not. The analysis of variance with stepwise regression, having an r^2 = 0.9272 ($P < 0.0001$), indicates the close association of these three organs, the predictive model for which is: Perineal gland mass = -18.670 + 0.443 (testes mass) + 0.398 (seminal vesicles mass).

Relationship of the perineal gland to reproductive organs

Pearson's correlation coefficient was used to assess the seasonal relationships of the perineal gland, testes, and seminal vesicles, including their relationships to other body measurements. Perineal gland weights were compared with body measurements and with weights of the body, testes, and seminal vesicles. All correlations except those with ear and hind foot measurements were highly ($P < 0.001$) correlated. The strongest correlations were between the perineal gland and the testes and seminal vesicles. The testes, producers of androgens, showed a positive and highly significant correlation with perineal gland weight ($r = 0.933$, $P < 0.001$). The seminal vesicles, secondary sex organs responsive to androgens produced by the testes and producers of seminal fluid, also showed a similar significant correlation to the perineal gland ($r = 0.930$, $P < 0.001$).

In order to more closely examine the changes in the perineal gland during the breeding season, perineal gland weights were compared to reproductive organ weights for monthly samples. Not only did the mean monthly weights of the perineal gland change dramatically throughout the year (Fig. 5), but the weights of the testes and seminal vesicles went through similar cyclical changes. Substantial growth in all three organs began in February. However, testes weight increased earlier and more rapidly than the related organs, increasing 13-fold from January to February. From February to March, testes weight increased only 1.8 times, whereas perineal gland and seminal vesicles increased 5- and 7-fold, respectively. Later, the changes in all three organs were more synchronous.

Weights of the perineal gland, testes and seminal vesicles all decreased in mean values during July (Fig. 5), probably due to the recruitment of spring-born males into the population. In August, the mean organ weights rebounded nearly to their previous high levels (with mean body weight also increasing from 87.5 g to 108 g), but then a rapid reduction in the sizes of all three organs was seen in October. Mean testes weight decreased sharply in October, and regression continued so that from November to January, the paired testes weights of all males ranged from 30-50 mg, and the related organs were tiny, if detectable. All three organs remained low in weight until the following February, when the cycle began anew.

Preliminary field studies

We made two attempts, only partly successful, to learn the information content of the product of the perineal gland by placing derivatives of the gland into the natural environment and evaluating the responses of wild cotton rats.

The first study, using a homogenate of perineal gland obtained from several adult males placed on filter paper pieces and randomly introduced as a treatment in half of the traps, resulted in a significant difference in the responses to treatment versus control traps: 26 males were captured in traps with the perineal gland homogenate but only 4 males in the control traps ($X^2 = 16.14$, $df = 1$, $P < 0.0001$). But no significant differences were seen in females (homogenate: $n = 11$ and control: $n = 9$).

The intriguing results of this first field test prompted a second field study the next autumn, when an attempt was made to learn more details of the chemical nature of the active component(s) in the secretion. Perineal gland homogenates and both fractions from a chloroform:methanol extraction of perineal gland homogenate were used. A homogenate from hind leg muscle of adult males and the extracted fractions from the muscle homogenate were used as controls. These six treatments (perineal gland homogenate with its chloroform and methanol fractions, muscle homogenate with its similar chloroform and methanol fractions, and controls) were placed in traps in the field as before. Despite 196 captures of cotton rats over 10 days of trapping, no homogenate or extract was better than any other at attracting or repelling either sex. Thus, the results of the second field study were inconclusive, and failed to give further clues as to the information content of the glandular product.

DISCUSSION

In a review of 20th century investigations on the role of chemical communication in rodents, Johnson (2003) reports that studies, mostly conducted on lab mice, indicate secretions usually are a mix of 50 or more different chemical compounds and that scent overmarking behavior reveals that many rodents can evaluate and respond to changes in their olfactory environment. In their study of scent communication in hispid cotton rats in Texas, Gregory and Cameron (1989) found that dominant males marked more often with urine than subordinates or females, but both sexes seemed to mark their environments with feces.

In order to determine the anatomy, method of delivery of secretory products, cyclical nature, and probable function of the perineal gland in *Sigmodon hispidus*, we examined several aspects of this previously undescribed organ. The subcutaneous location and close association of the perineal gland to the rectum and penis (Figs 1 and 2) indicated three possible routes of delivery for any glandular products: through the skin, in feces, or via the urethra, all used by rodents. Although many species of arvicoline rodents possess enlarged sebaceous glands whose products are extruded onto specialized hairs or skin pads, as in the caudal gland of *Dicrostonyx groenlandicus* (Quay, 1968), the large size of the gland in *Sigmodon*, a sigmodontine rodent, the presence of only loose fascial attachments to the skin, and the lack of skin or hair specializations near the gland suggested no delivery through the skin. In addition, the flimsy fascial attachments to the

rectum made a fecal delivery (as occurs in *Microtus agrestis*— Khan, 1984, or in *Thrichomys aperoides*—Talamoni et al., 2014) doubtful. The third possible route, urethral delivery through the urinary tract, as seen in the beaver (Svendsen, 1978) or in three *Rattus* species in Australia (Mallick, 1992), seems the most plausible delivery route of glandular products. In fact, dissection revealed strong ligamentous attachments of the gland to the penis, further supporting the hypothesis of a close association of the perineal gland to the reproductive and lower urinary tract. However, no duct connecting the gland to the urethra could be found during the gross dissection of several males.

During necropsy, a distinctive pungent odor was associated with the developed perineal gland of breeding males. A similar odor often emanated from live traps or was detected when handling reproductive males in the field, to the extent that the sex of the animal could accurately be predicted before examining the genitalia.

A histological study of maturing males, i.e., those with partially enlarged perineal glands, supported the notion of a urinary delivery route. Several difficulties arose when preparing tissues for histology, relating to the oily or waxy texture of the gland, the massive size of a fully enlarged gland, and the large blocks of pelvic regions that would be needed to confirm attachment or association of other organs. However, one interpretable series of cross sections of the perineal region was obtained from the area near the attachment of the perineal gland to the penis. Although no connections or attachments between the perineal gland and the rectum or skin were seen, an attachment to the penis was evident. A neck-like projection at the end of a bulb-shaped section of the gland extended towards the penis. The medial end formed a glandular mass that abutted the muscular band of the penis, and glandular tissue extended into the penis toward the urethra.

The entire gland is surrounded by an investment of smooth muscle (see Figs 3 and 4) and the septated multilobular acinar bundles within the bulb of the gland contain small tubules which coalesce into a large tubule or duct through the neck-like portion of the gland. Together with evidence of a tubule within the muscular band of the penis near the urethra, these findings suggest an active method of delivery (controlled by smooth muscle contractions) that probably injects the product of the gland through a duct leading into the urethra and the urine. Further histology is needed to confirm the existence of this ductal opening into the urethra and to determine the nature of the histological changes that occur at different phases of the male reproductive season. For the present, a urethral entry of glandular products and placement via the urine seems most likely.

Seasonality of the perineal gland

The cyclicity of the perineal gland corresponds closely with the seasonal weight changes of the reproductive organs (Figure 5). As with the responses of testes and seminal vesicles to the approaching breeding season, the perineal gland enlarges from a tiny organ that cannot be dissected away from the surrounding tissues to a large oily gland whose mass comprises up to 1.4% of the total body weight in some males. Together with the testes (up to 1.8%) and seminal vesicles (up to 1.6%), these organs can

comprise up to 4.8% of the total body weight of large males and thus their enlargement requires substantial energy costs, for growth before and for maintenance during the breeding season. In contrast, during the non-breeding seasons these three organs might sum to 75 mg at the most, which is less than 0.01% of body weight for a 100-g male. A 90% reduction in energy costs for organ maintenance is likely to be associated with the regression of these three organs. Because seasonal regression of the sex organs serves as an energy-saving adaptation in small mammals to enhance winter survivability (e.g., Blank, 1992), the regression of the perineal gland would further increase these energy savings. However, this additional energy reduction can be adaptive only if the necessary products or functions of the perineal gland are no longer needed during the non-breeding months of winter. Thus, the regression of this gland in late autumn further indicates that the perineal gland and its secretions serve reproduction in some capacity.

The synchronous regression and recrudescence of perineal gland and seminal vesicles suggests that both are under androgen control. Because androgens are required for growth and maintenance of accessory sex organs, for external secondary sexual characteristics, and for some pheromone-producing glands (Bronson, 1989), we would expect (and saw—Fig. 5) the growth of the testes to precede the enlargement of the seminal vesicles and perineal gland. The mean weight of testes showed a dramatic increase in February, whereas the increases in mean weights of the perineal gland or seminal vesicles, small in February, were not substantial until March (Fig. 5). The converse was true at the end of the breeding season in October, when the weights of seminal vesicles and perineal gland dropped dramatically while the testes weights declined more gradually. A similar pattern of growth and regression in the preputial gland of muskrats was shown by Beer and Meyer (1951) to be under androgen control. Seminal vesicles have been shown to be under androgen control in other rodents (e.g., Tamarkin et al., 1976), so their enlargement is expected after testicular recrudescence, with its related increase in androgen production. Likewise, the regression of seminal vesicles occurs as androgen production drops in autumn (before the reduction in testes weight). Because changes in weights are synchronous with those of the seminal vesicles, we believe that the perineal gland is under the same androgen control as the seminal vesicles. Further support for this hypothesis is seen in the androgen control of specialized sebaceous glands. In several families of mammals, glandular reduction by castration and either growth or maintenance of the gland after castration by androgen therapy have been demonstrated in the anal and chin glands of rabbits (Mykytowycz, 1965), in the proctodeal gland of the short-tailed vole (Khan, 1984), in the supracaudal gland of the guinea pig (Martan, 1962), and in the ventral gland of the Mongolian gerbil (Thiessen et al., 1968). The perineal gland in *S. hispidus* probably is another example.

The only deviation from the spring increase and fall decrease in organ weights occurred as a dip in the mean weights of all three organs in July (Fig. 5). In his studies of the kangaroo rat, *Dipodomys merriami*, a species with sexual dimorphism in a dorsal skin gland, Quay (1953) observed a similar pattern when the mean area of the gland showed a drop in May but recovery in June. The decreases in mean organ weights during July in *S. hispidus* and in May in *D. merriami* corresponded to the times when spring-born animals of both species are first entering the reproductive populations. This introduction of

young, small males undergoing their sexual maturation would tend to decrease the mean weights for the month, thereby explaining the July dip in *S. hispidus.* In brief, the July decreases in mean organ weights are not due to real reductions in organ weights of individuals but are explained by the changing composition of the population as young-of-the-year males entered the breeding population.

Features of the anatomy, preliminary histological studies and similarities in patterns of growth and regression strengthen the evidence of a link between the perineal gland and reproductive functions in male cotton rats. Statistical analyses confirmed these relationships. All three organs (testes, seminal vesicles, perineal gland) showed significant differences of the mean organ weights of males between breeding and non-breeding seasons. In addition, high correlations of the perineal gland to those of the testes and seminal vesicles (both $P < 0.001$) indicated a high probability for the gland to be associated with the reproductive system. Finally, the linkage of the pattern of recrudescence and regression of the perineal gland to that of the reproductive organs (Fig. 5) was further illustrated by the significant results from the ANOVA with stepwise regression.

The field study using the exudate of whole perineal glands produced significant exploratory behavior in males but not in females, perhaps suggesting that males were guarding females or seeking to find and expel other males from their territories. When the exudate was divided into polar and non-polar portions and these odors were placed in traps, no responses were seen in either sex in the second field study. In brief, more lab and field studies are needed to determine the nature and function of the chemicals produced by the perineal gland, and their role in normal reproduction.

In conclusion, the perineal gland showed seasonal cycling and a strong relationship to the reproductive system in the male hispid cotton rat, *Sigmodon hispidus.* The gland, probably under the same androgen control as the seminal vesicles, enlarges from an indiscernible organ to one weighing up to 1.4% of total body weight in large breeding males. The energy expenditure for growth and maintenance of the perineal gland at a time when reproductive energy requirements are great suggests the importance of the gland and the necessity for its products as part of successful reproduction. Our studies suggest that the glandular product is deposited into the environment in the male's urine. Perhaps this product signals the presence of a reproductive male and serves as a territorial marker, but its message is still unclear.

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Table 1. Means (± *SE*) and weights (mg) of perineal gland, testes, seminal vesicles, and body, and linear measurements (mm) for adult male cotton rats (> 50 g) collected each month throughout the year. The non-breeding season includes the months of November through January (when the perineal gland is too small to detect) and the breeding season extends from February through October. Body length is total length minus tail length.

	Annual *N* = 129		Non-breeding season *N* = 39		Breeding season *N* = 90	
Variables	Mean	Range	Mean	Range	Mean	Range
Perineal gland	568 ± 55.7	1-2342	1.0 ± 0.0	0	813 ± 65.2	1-2342
Testes	776 ± 63.6	20-2311	44.5 ± 2.7	20-104	1093 ± 68.4	38-2311
Seminal vesicles	579 ± 64.6	1-3080	6.8 ± 0.8	1-15	827 ± 80.0	1-3080
Body weight	97 ± 2.3	51-168	88.3 ± 3.2	52-121	101 ± 2.8	51-168
Body length	145 ± 1.3	116-185	137.3 ± 1.8	114-160	148 ± 1.7	116-185
Hind foot length	30.2 ± 0.1	26-35	29.9 ± 0.3	26-35	30.1 ± 0.1	26-35
Ear length	17.8 ± 0.1	14-21	17.9 ± 0.1	15-19	17.7 ± 0.1	14-21

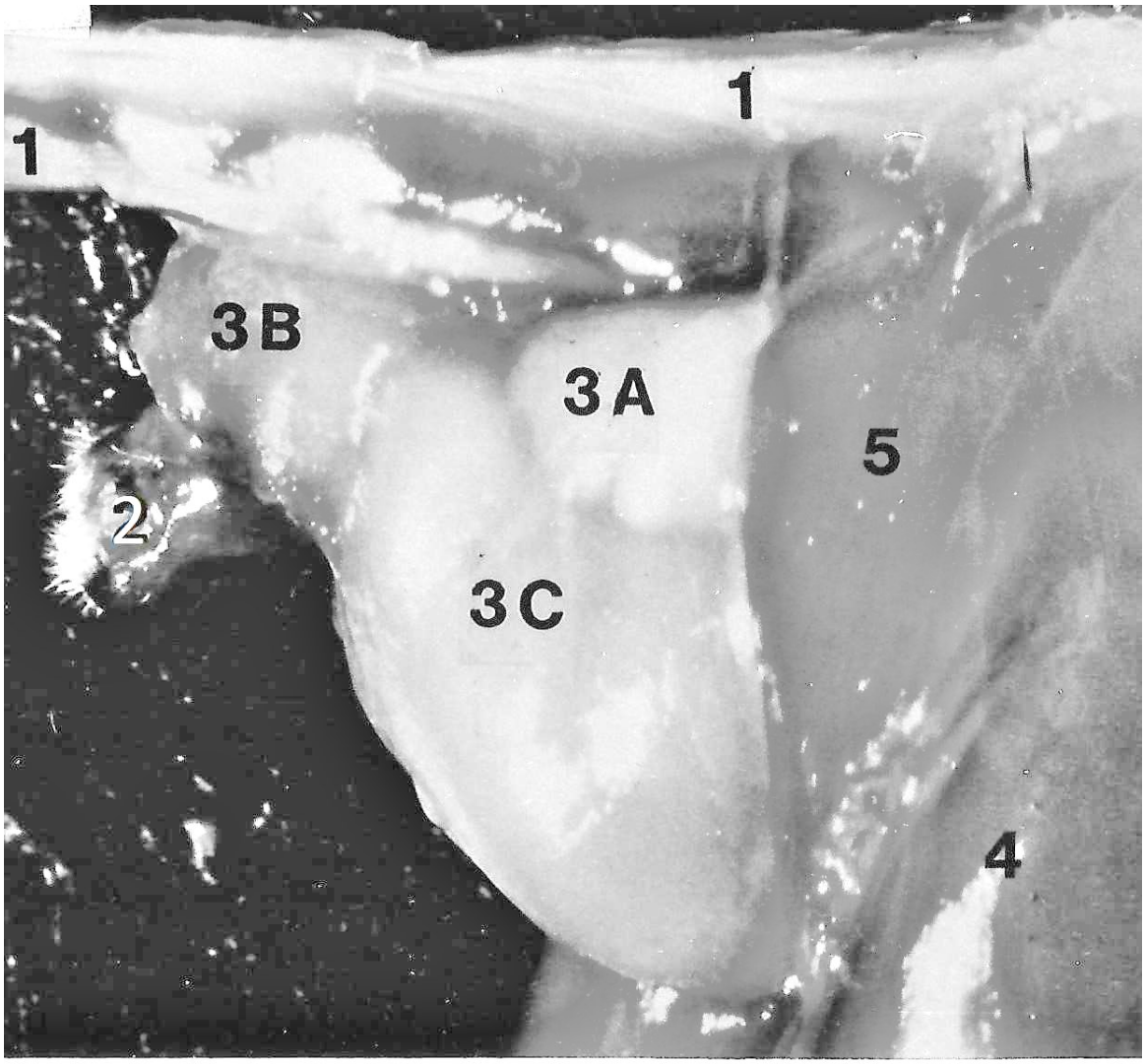

Figure 1. The location of a fully developed perineal gland in the male hispid cotton rat, *Sigmodon hispidus*. The testes have been removed to enable the parts lying dorsal to the testes to be identified: tail (1), anus at end of rectum (2), the three right lobes of the perineal gland (3A, 3B, 3C), and skeletal muscles of the thigh (4, 5).

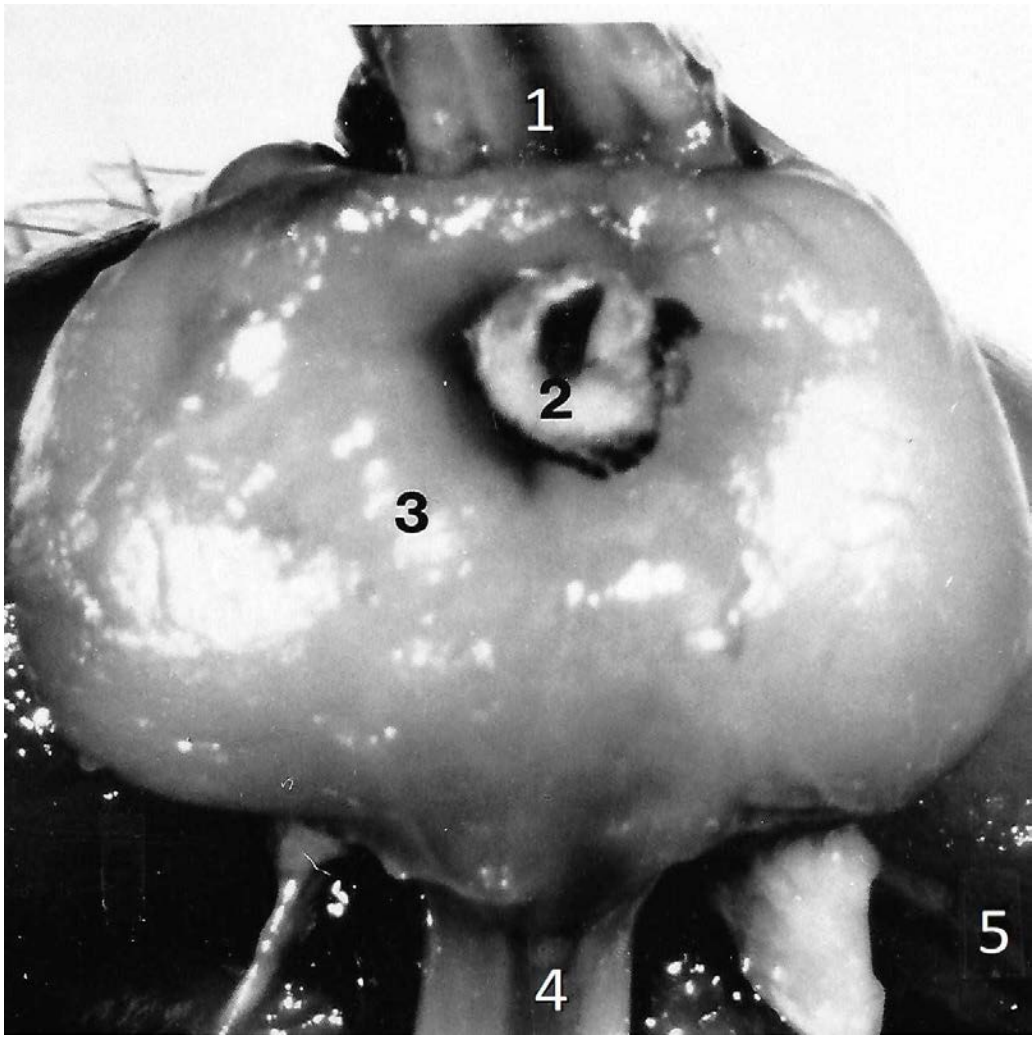

Figure 2. A posterior view of the perineal gland showing the tail (1), cross section of rectum (2), perineal gland (3), penile shaft (4) and muscle of the thigh (5).

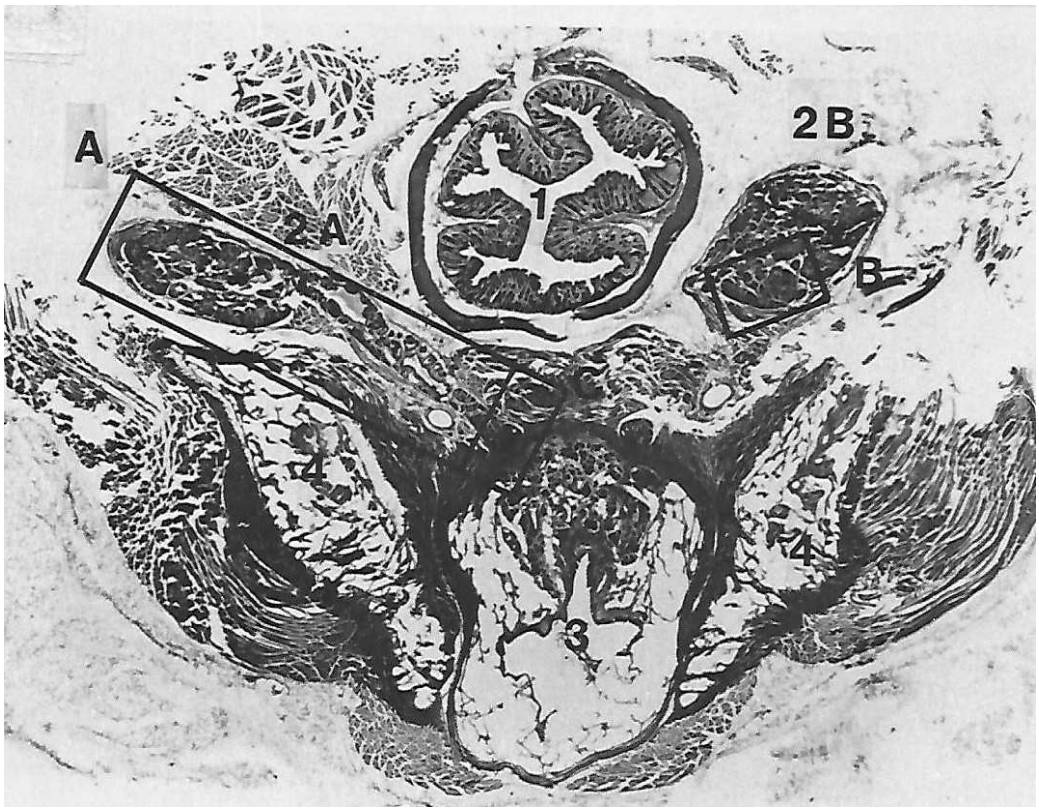

Figure 3. Histological cross-section of the pelvic region of a mature male cotton rat showing the rectum (1), regions of the perineal gland (2), urethra (3), other regions of the perineal gland (4).

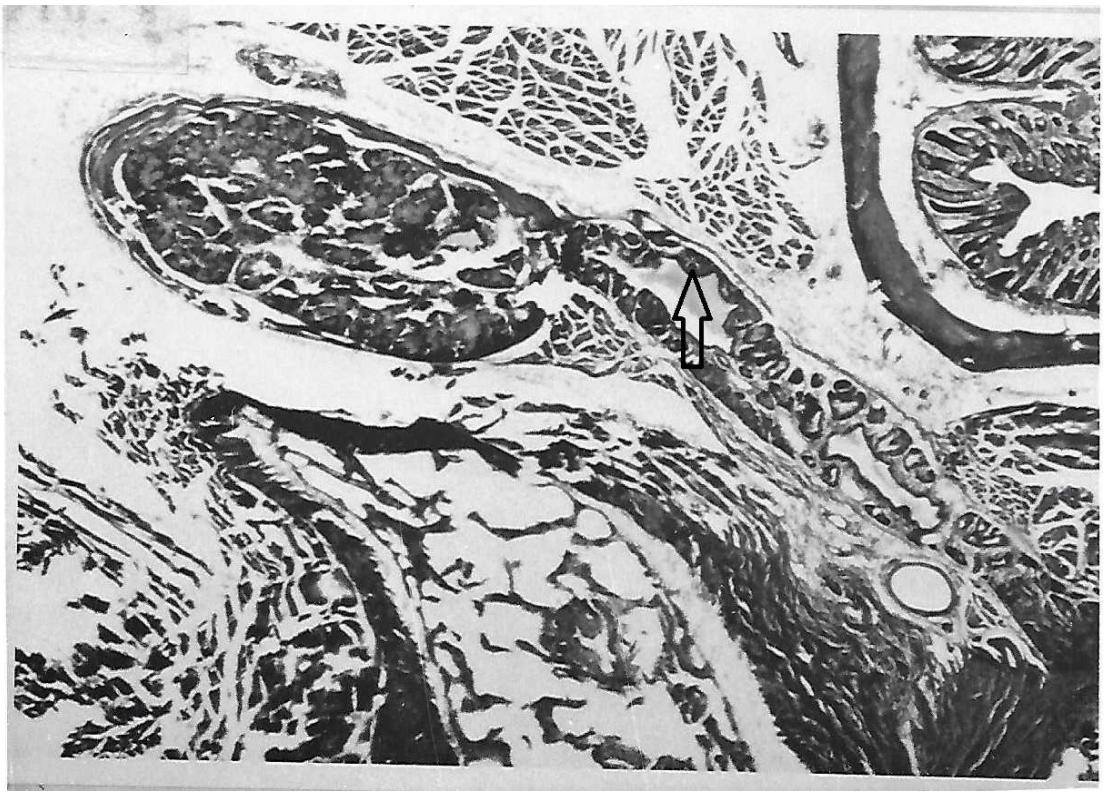

Figure 4. Higher magnification of the rectangular “A” region of Figure 3, showing the acinar cells in the bulbous secretory area of a lobe of the perineal gland, and the neck-like middle section (with arrow pointing to an acinar cell) ending in a tube extending towards the penis.

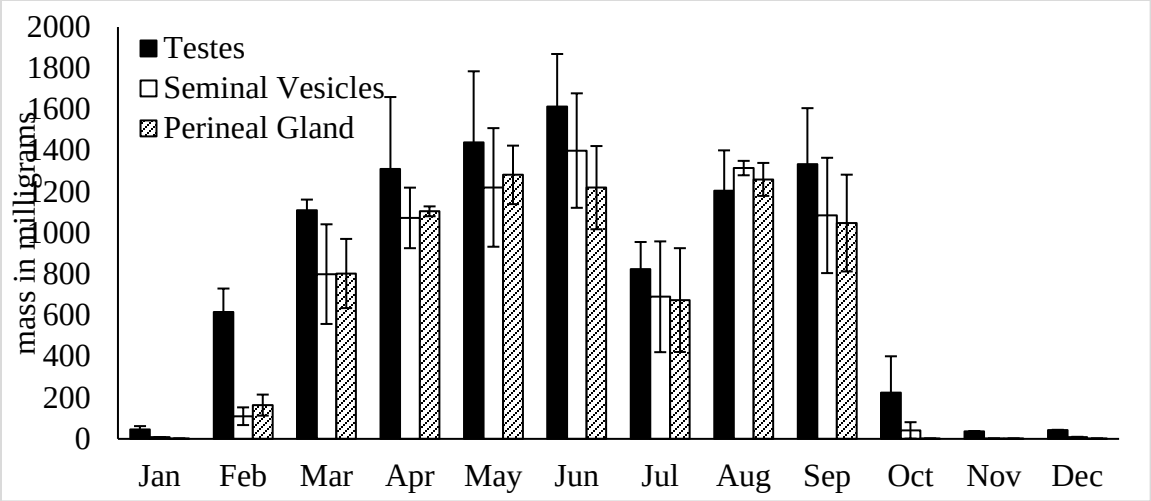

Figure 5. Comparison of mean monthly weights ± *SE* of paired testes, seminal vesicles, and perineal gland. $n = 129$. Notice that testes mass almost always is greater than the others, except for August, which signals rapid changes to come in autumn.