

American Museum Novitates

PUBLISHED BY THE AMERICAN MUSEUM OF NATURAL HISTORY
CENTRAL PARK WEST AT 79TH STREET, NEW YORK, N. Y. 10024

NUMBER 2532

JANUARY 23, 1974

Comparative Morphology of Spermatozoa of the Rodent Genus *Peromyscus* (Muridae)

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ABSTRACT

The morphology of spermatozoa of 27 species and seven subgenera of the genus *Peromyscus* was studied. Measurements of length and width of the head, midpiece length, and tail length of the sperm of 25 species are given. Two major sperm types occur in the series. Sperm of two species of the subgenus *Habromys* examined have large heads lacking the hooklike process characteristic of many murid rodents, whereas all other species studied have sperm with hooked heads. Although there are species differences in shape of the head and of various measurements of the sperm among the group with hooked heads, no definite patterns of variation corresponding to presently recognized subgenera or to the species groups within the subgenus *Peromyscus* were seen. There is no obvious correlation between body size and sperm size.

INTRODUCTION

Morphology of the reproductive system has provided useful taxonomic criteria in many groups of mammals. In a previous study (Linzey and Layne, 1969) we found that variation in the accessory sex gland complement and other features of the male genital tract of *Peromyscus* was valuable in assessing relationships of the species currently assigned to this large and diverse genus of rodents. As an outgrowth of this work, we have examined

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morphology of the spermatozoa of *Peromyscus* to determine the extent to which variation in the mature gametes reflects the structural patterns of the male reproductive apparatus.

Bishop and Austin (1957) suggested that the sperm of each mammal species is probably unique. However, relatively few systematic surveys of sperm variation within particular taxa have been undertaken. The principal studies of this type include those of Hughes (1965) on Australian marsupials, Biggers (1966) on Neotropical marsupials, Forman (1968) on North American vespertilionid and phyllostomatid bats, Hirth (1960) on North American vespertilionid bats and rodents, Friend (1936) on British murid rodents, and Illisson (1971) on Australian and New Guinea murid rodents. Spermatozoa of five species of *Peromyscus* and *Ochrotomys nuttalli*, formerly included in the genus, have been described by Hirth (1960).

MATERIALS AND METHODS

We examined sperm of 27 species representing all the subgenera recognized by Hooper (1968). Sperm preparations were made by teasing apart small pieces of cauda epididymal tubules in several drops of distilled water on a microslide. The fragments of tissue were then removed and the slide allowed to dry. Several slides were usually prepared from each specimen. In each series, some preparations were left unstained for examination, and others were stained with Heidenhain's iron haematoxylin. Slides were either mounted in Permount or kept unmounted for study. In a few instances sperm were obtained from freshly killed specimens, but the majority of the preparations were made from specimens originally fixed in 10 percent formalin and stored for varying periods of time in 70 percent alcohol. No differences were noted in the morphology or measurements of sperm from fresh or preserved specimens of the same species. The only artifacts of preparation appeared to be occasional distortion of the position of the hook and breakage of the tail.

Sperm were studied with ordinary light and phase contrast microscopes at magnifications ranging from $\times 100$ to $\times 1070$. The following measurements, in microns, were taken with an ocular micrometer: (1) maximum length of head; (2) greatest width of head, excluding the hook if present; (3) length of midpiece; and (4) length of tailpiece. The last measurement was most subject to error because of the usual curvature of the tail and the difficulty of detecting cases in which small pieces of the end were missing. For purposes of description and measurement, the sperm head was considered to be dorsoventrally flattened, a convention followed by Forman (1968).

ACKNOWLEDGMENTS

We are deeply grateful to the following individuals and institutions for providing specimens for the present study: Drs. Dale E. Birkenholz, Leonard Brand, Daniel S. Fertig, Charles O. Handley, Jr. (National Museum of Natural History, Smithsonian Institution), Emmet T. Hooper (Museum of Zoology, University of Michigan), Guy G. Musser, and Andrew Starret. We are also indebted to Mr. David G. Huckaby for clarifying identifications of certain specimens used in this and our previous study of the genital tracts. The present study was supported in part by National Science Foundation Grant GB-4174.

RESULTS

Spermatozoa of all except two species of *Peromyscus* examined were of the same basic type. In *Peromyscus* the flattened sperm head is typically asymmetrical, with one margin smoothly convex and the other straighter. The base is truncated and often has a sinuous margin. The anterior portion of the head is enveloped by the acrosome cap and is extended into a sharp, strongly recurved hook that lies on the side of the head with the straighter margin. The head is strongly emarginate at the base of the hook on the side to which the hook bends. The base of this emargination is a shoulder-like projection formed by a clear flap of acrosomal material referred to as the "dorsal eave" by Friend (1936). The degree of development of this structure varies among species. In stained preparations, a more deeply stained area in the posterior region of the head, the "dense posterior region" of Friend (1936), is often discernible.

The midpiece is distinctly set off from the head by its narrower width. Its point of attachment to the base of the head is usually displaced toward the side on which the hook is situated, although in those species where the base of the head is narrow, the midpiece tends to be more centrally attached. Some sperm possess a bulge on the distal portion of the midpiece similar to that figured by Hirth (1960) for the bat *Lasiurus borealis* and several species of rodents, including *Sigmodon hispidus*, *Microtus ochrogaster*, *Neofiber alleni*, and *Mus musculus*. This "cytoplasmic droplet" is a developmental feature (Bishop and Austin, 1957) and therefore is of no value for comparative purposes.

The tailpiece is relatively clearly delimited from the midpiece. It is broadest at the proximal end and tapers gradually toward the tip.

In the two species of the subgenus *Habromys* examined, the spermatozoa differed from the general type described above, the major difference being the absence of a hook.

Absolute and proportional measurements of the sperm of all but two of

the species studied are given in table 1. The means and ranges, in microns, of measurements of all species are as follows: head length, 5.5 (5.1–6.2); head width, 3.0 (2.4–4.9); length of midpiece, 17.5 (15.2–21.4); length of tailpiece, 56.0 (49.3–69.3). Relative size of the various components expressed as a percentage of total sperm length are as follows: head length, 7.0 (5.4–8.1); head width, 3.8 (2.8–6.4); midpiece length, 22.2 (19.9–25.7); tailpiece length, 70.9 (66.2–73.8).

SPECIES DESCRIPTIONS

SUBGENUS *PEROMYSCUS*

Maniculatus GROUP

Peromyscus polionotus

SPECIMENS EXAMINED: Four. Florida: Alachua County (3); St. Johns County, Anastasia Island State Park (1).

The sperm head is rounded, tapering abruptly to its apex. It is widest at the middle, and the base is relatively broad. The hook is short and strongly recurved, extending back about one-third the length of the head. The dorsal eave is not well developed. The dense posterior region makes up approximately one-third of the area of the head. The midpiece is short and its attachment highly eccentric. The midpiece is proportionally shorter and the tail longer than the average of all species.

Peromyscus maniculatus

Figure 1A

SPECIMENS EXAMINED: Nineteen. New York: Jefferson County (3). North Carolina: Swain County (14). Tennessee: Cocke County, Cosby (2).

The sperm head is shorter than average and of uniform width; the anterior portion is only slightly tapered, hence the hook has a broad base. The hook is long and strongly recurved, extending back over one-half of the length of the head. The dorsal eave is prominent, and the dense posterior region is restricted to a small area just anterior to the base. The base is broad, and the midpiece attaches to one side. Relative length of the midpiece approximates the average of all species, whereas the tail is proportionally longer than average.

Peromyscus melanotis

SPECIMEN EXAMINED: One. Mexico: Distrito Federal, LaCima, 143 km. south of Mexico City.

The sperm head is slightly longer and broader than average in relative

size, although it is close to the mean in actual size. It is widest at the middle, tapering gradually to the base of the hook, which is narrow. The long, strongly recurved hook extends back from one-half to two-thirds of the length of the head. The dorsal eave is well developed, and the dense posterior region occupies approximately the basal half of the head. In the specimens examined, the midpiece attachment varies from nearly central to highly eccentric. The midpiece is longer than average in relative length, and the tail is shorter.

Leucopus GROUP
Peromyscus leucopus

SPECIMENS EXAMINED: Eight. Tennessee: Cocke County, Cosby.

The sperm head is very broad and rounded, widest just above the middle, with the distal portion tapering abruptly. The hook is short and strongly recurved, extending back about one-third the length of the head. The dorsal eave is only slightly developed. The dense posterior region is triangular, with the anteriormost projection extending to the broadest portion of the head on the side with the hook. The dense posterior region comprises about one-third of the total area of the head. The base of the head is broad, and the midpiece attaches to one side. The proportional sizes of the midpiece and tail are close to the mean of all species.

Peromyscus gossypinus

Figure 1B

SPECIMENS EXAMINED: Four. Florida: Alachua County, Gainesville (2). Tennessee: Cocke County, Cosby (2).

The sperm head is narrower and less rounded than that of *P. leucopus*, widest toward the base, tapering gradually to the origin of the hook. The hook is short and strongly recurved, extending back about one-third the length of the head. The dorsal eave is prominent. The dense posterior region comprises 25 percent of the area of the head. The base is broad. The midpiece attaches eccentrically and, in actual measurement, is slightly longer than that of *P. leucopus*. Proportionally, the midpiece is shorter than that of *P. leucopus*, whereas the tail is longer.

Crinitus GROUP
Peromyscus crinitus

Figure 1C

SPECIMEN EXAMINED: One. California: Deep Springs.

The sperm head is of average length and width, broadest at the middle,

TABLE 1

MEANS AND STANDARD ERRORS OF MEASUREMENTS AND RELATIVE SIZE OF VARIOUS COMPONENTS OF SPERM OF 25 SPECIES OF *Peromyscus*
(N = 10 except as noted)

Subgenus and Species	Measurements (microns)				Relative Size (percentage of mean total length)			
	Head Length	Head Width	Midpiece Length	Tail Length	Head Length	Head Width	Midpiece Length	Tail Length
Subgenus <i>Peromyscus</i>								
<i>poilonotus</i>	5.4±.05	3.0±.13	15.2±.13	54.2±.64 ^a	7.2	4.0	20.3	72.5
<i>maniculatus</i>	5.2±.08	3.0±.05	16.7±.08	53.1±1.53	6.9	4.0	22.3	72.3
<i>melanotis</i>	5.3±.07	3.1±.08	16.7±.10	51.3±.71	7.2	4.2	22.8	70.0
<i>leucopus</i>	5.3±.24	3.4±.16	16.8±.00	52.7±1.21	7.1	4.5	22.4	70.4
<i>gossypinus</i>	5.4±.00	2.9±.09	17.3±.04	63.4±1.11	6.3	3.4	20.1	73.6
<i>crinitus</i>	5.2±.08	2.8±.08	19.0±.23	54.6±.83	6.6	3.6	24.1	69.3
<i>boylei</i>	5.5±.05	2.4±.07	17.0±.08	55.1±1.42	7.1	3.1	21.9	71.0
<i>truei</i>	5.4±.05	2.6±.07	16.2±.08	56.8±.94	6.9	3.3	20.7	72.4
<i>difficilis</i>	5.4±.07	2.6±.07	17.0±.08	56.4±1.24	6.8	3.3	21.6	71.6
<i>mexicanus</i> ^b	5.5±.19	2.6±.10	17.4±.24	49.3±3.62	7.6	3.6	24.1	68.3
<i>nudipes</i>	5.5±.07	2.6±.08	17.1±.08	56.6±1.41	6.9	3.3	21.6	71.5
<i>furus</i>	5.3±.07	2.5±.00	17.1±.11	54.8±2.31 ^b	6.9	3.2	22.2	71.0
<i>guatemalensis</i>	5.5±.05	2.6±.07	18.2±.08	58.5±1.18	6.7	3.2	22.1	71.2
<i>megalops</i>	5.1±.07	2.6±.07	19.5±.15	69.3±2.01	5.4	2.8	20.8	73.8
<i>zarynchus</i>	5.2±.08	2.5±.00	17.7±.11	52.5±1.89 ^c	6.9	3.3	23.5	69.6
<i>grandis</i>	5.3±.07	2.5±.00	17.8±.13	56.7±.47	6.6	3.1	22.3	71.0

TABLE 1—(Continued)

Subgenus and Species	Measurements (microns)			Relative Size (percentage of mean total length)				
	Head Length	Head Width	Midpiece Length	Tail Length	Head Length	Head Width	Midpiece Length	Tail Length
Subgenus <i>Haplomydomys</i> <i>californicus</i> <i>eremicus</i>	5.3±.08	3.4±.10	15.2±.16	51.5±2.54	7.4	4.7	21.1	71.5
	5.9±.12	3.5±.05	16.6±.08	53.5±2.42	7.8	4.6	21.8	70.4
Subgenus <i>Osgoodomys</i> <i>banderanus</i>	5.6±.08	2.7±.08	18.0±.11	56.0±.99	7.0	3.4	22.6	70.4
	5.2±.13	2.9±.10	15.9±.18	53.9±1.45	6.9	3.9	21.2	71.9
Subgenus <i>Isthomys</i> <i>flavicus</i> <i>pirrensis</i>	5.9±.00	3.0±.00	16.8±.00	61.3±1.24	7.0	3.6	20.0	73.0
	5.8±.07	2.9±.05	16.9±.05	62.2±1.10	6.8	3.4	19.9	73.3
Subgenus <i>Megadontomys</i> <i>thomasi</i>	5.7±.13	3.1±.07	21.4±.26	62.9±1.40	6.3	3.4	23.8	69.9
	6.0±.15	4.1±.17	19.3±.20	51.8±.62	7.8	5.3	25.0	67.2
Subgenus <i>Habromys</i> <i>lepturus</i> <i>lophurus</i>	6.2±.14	4.9±.15	19.7±.13	50.8±.76	8.1	6.4	25.7	66.2

°N = 8. °N = 5. °N = 9.

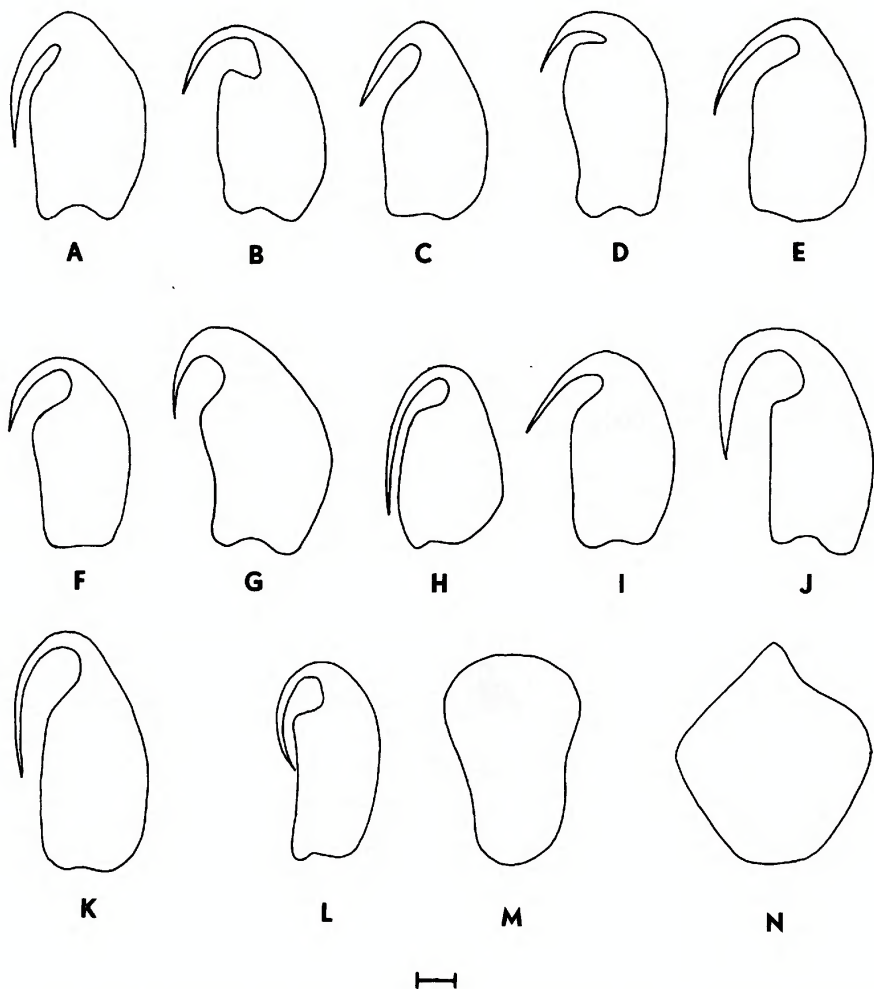


FIG. 1. Heads of spermatozoa of representative species of the subgenera and species groups of *Peromyscus*. A. *P. maniculatus* (subgenus *Peromyscus*, *Maniculatus* group). B. *P. gossypinus* (subgenus *Peromyscus*, *Leucopus* group). C. *P. crinitus* (subgenus *Peromyscus*, *Crinitus* group). D. *P. boylei* (subgenus *Peromyscus*, *Boylei* group). E. *P. truei* (subgenus *Peromyscus*, *Truei* group). F. *P. guatemalensis* (subgenus *Peromyscus*, *Mexicanus* group). G. *P. eremicus* (subgenus *Haplomydomys*). H. *P. banderanus* (subgenus *Osgoodomys*). I. *P. floridanus* (subgenus *Podomys*). J. *P. pirrensis* (subgenus *Isthmomys*). K. *P. thomasi* (subgenus *Megadontomys*). L. *P. lepturus* (subgenus *Habromys*), hooked form. M. *P. lepturus*, typical form. N. *P. lophurus* (subgenus *Habromys*). Scale equals 1 micron.

tapering gradually to the base of the hook. The hook is of moderate length, extending back slightly more than one-third the length of the head. The dorsal eave is well developed. The dense posterior region is confined to a small area adjacent to the base of the head. The base is relatively narrow compared with that in such species as *P. leucopus* and *P. gossypinus*. The midpiece is long both in actual and relative size and attaches eccentrically to the head. The tail is proportionally shorter than average.

Boylei GROUP
Peromyscus boylei

Figure 1D

SPECIMENS EXAMINED: Three. Guatemala: Department of Quezaltenango, 2 miles north of Quezaltenango (1). Arizona: Cochise County, Chiricahua Mountains (2).

The sperm head is of average length but narrow, being widest just above the base. The basal portion is squarish, with the anterior portion tapering only slightly. The hook, which originates from a broad base, is short and weakly recurved. The dorsal eave is not prominent. In stained specimens, no clearly differentiated dense posterior region was observed, the entire head being lightly stained. The base of the head is broad, and the midpiece attaches eccentrically. Relative proportions of the midpiece and tail are close to the average for all species.

Peromyscus oaxacensis

SPECIMEN EXAMINED: One. Mexico: Chiapas, 1 mile north of Pueblo Nuevo.

The sperm head is oblong and widest above the middle, tapering abruptly so that the hook origin is narrow. The hook extends anteriorly for a short distance before curving backward. The dorsal eave is not prominent. In stained specimens, the entire sperm head was lightly stained except for darkly pigmented areas at the apex and in the extreme basal area. The base of the head is relatively narrow, the attachment of the midpiece being nearly central. No measurements of the sperm of this species are available.

Truei GROUP
Peromyscus truei

Figure 1E

SPECIMENS EXAMINED: Two. Colorado: Boulder County, Boulder (1). Mexico: Oaxaca, Tlacolula (1).

The sperm head is close to average in length but narrow, broadest near the base with the anterior portion narrowing abruptly. The hook, which has a narrow base, is of moderate length and strongly recurved. The dorsal eave is prominent. The dense posterior region covers about a quarter of the area of the sperm head. The base is of medium width, and the midpiece attaches slightly to one side of the center. The midpiece is slightly shorter than average in relative length, and the tail is longer than average.

Peromyscus difficilis

SPECIMEN EXAMINED: One. Mexico: Oaxaca, Cerro San Felipe.

The sperm head is approximately the same size as that of *P. truei* and oblong in shape, widest at the middle, and tapering gradually to the narrow hook origin. The hook is very short and only slightly recurved, barely extending backward past the apex of the head. The dorsal eave is prominent. In stained preparations, the head is uniformly stained except for a dark line extending along the base and slightly up the sides. The base of the head is wider than that of *P. truei*, and the midpiece attachment is strongly eccentric. In relative size, the midpiece is slightly shorter and the tail slightly longer than average.

Mexicanus GROUP

Peromyscus mexicanus

SPECIMEN EXAMINED: One. Mexico: Chiapas, El Suspiro.

The sperm head is of average length but narrow, the widest part being near the middle. The base is fairly broad, and the margin opposite the hook is not strongly curved. The origin of the hook is narrow, and the hook extends to the middle of the head or slightly beyond. The dorsal eave is well developed. In stained specimens, the head is evenly and densely stained throughout, except for a heavier line along the margin of the base. No clearly defined dense posterior region is evident. The midpiece attaches eccentrically and is relatively longer than average. The tail is proportionally short.

Peromyscus nudipes

SPECIMENS EXAMINED: Three. Costa Rica: Cartago (2). Panama: Chiriqui (1).

The sperm head is of average length but slightly narrower than average in width, widest at about the middle, and tapering gradually to the base of the hook. The hook is of moderate length, extending backward about one-third the length of the head. The dorsal eave is not prominent. In

stained material, the intensity of the stain of the head deepens from the apex to the base but there is no well-defined dense posterior region. In some sperm, the base of the head is relatively broad and the midpiece attaches to one side. In others the base is narrower and the midpiece attachment is nearly central. The relative length of the midpiece is slightly less than average, and the tail is slightly longer than average.

Peromyscus fuvvus

SPECIMENS EXAMINED: Two. Mexico: Puebla, 5.7 and 7.3 road miles southwest of Huauchinango.

The sperm head approximates that of *P. nudipes* in dimensions. The hook is relatively long, extending approximately one-half the length of the head. The dorsal eave is conspicuous. The dense posterior region is confined to the extreme basal area of the head. In the specimens examined there was variation in the width of the head, and the midpiece attachment varied from central to eccentric. Relative lengths of the midpiece and tail are close to the respective averages of all species combined.

Peromyscus guatemalensis

Figure 1F

SPECIMENS EXAMINED: Two. Guatemala: Department of Huehuetenango, 27 miles north of Chiantla (1); Department of Solloa, 3.2 miles east of Panajachel (1).

The sperm head is average in length but slightly narrow, broadest near the base, tapering gradually to a very narrow hook origin. The hook, strongly recurved, extends backward over approximately one-third of the head length. The dorsal eave is conspicuous. The dense posterior region is extensive, comprising about half the head area. The darkest staining area is in a line along the base. In most specimens, the base of the head is narrow and the midpiece attaches centrally. In some, however, the base is wider and the midpiece attachment is offset to one side. The sperm of this species is comparatively long. The midpiece is of average relative length, whereas the tail is proportionally slightly longer than average.

Peromyscus megalops

SPECIMEN EXAMINED: One. Mexico: Guerrero.

The sperm head is short and narrow, with the widest point at about the middle. The hook extends forward for a short distance before curving sharply posteriad. The dorsal eave is well developed. In stained preparations, the entire head was heavily stained, although more lightly anteriorly than posteriorly. There was, however, no well-defined dense

posterior region. The base of the head is very narrow, and the midpiece attaches to one side. Although the midpiece is unusually long in actual length, it is relatively shorter than average. The tail of *P. megalops* sperm is relatively and absolutely longer than that in any species examined. The sperm of this species also exceeds all others in total length.

Peromyscus zarhynchus

SPECIMEN EXAMINED: One. Mexico. Chiapas, 1 mile north of Pueblo Nuevo.

The sperm head is short and narrow, widest at the middle, tapering gradually to the narrow hook origin. The hook extends forward a short distance before curving backward. The dorsal eave is conspicuous. The dense posterior region was not sharply demarcated in stained sperm, there being a gradual increase in density of staining from apex to base of the head. The base of the head is narrow, and the midpiece attachment is almost central. The midpiece is proportionally slightly longer and the tail relatively shorter than average.

Peromyscus grandis

SPECIMEN EXAMINED: One. Guatemala: Department of Baja Verapaz, 12.5 miles north of Salama.

The sperm head is short and narrow, broadest near the base, tapering gradually from that point to the apex. The hook is very long and strongly recurved, extending backward about two-thirds the length of the head. The dorsal eave is prominent. The basal half of the head of stained sperm was darker than the anterior half, but no clearly defined dense posterior region was observed. The base of the head is narrow, and the midpiece attachment is central. Proportional lengths of the midpiece and tail are close to the average of all species studied.

SUBGENUS *HAPLOMYLOMYS*

Peromyscus californicus

SPECIMEN EXAMINED: One. California: Riverside County, 2 miles from Corona.

The sperm head is slightly shorter and wider than average; the sides are nearly parallel until close to the apex, where they taper abruptly. The hook is long and strongly recurved, extending back one-half to two-thirds the length of the head. The dorsal eave is prominent. The dense posterior region is restricted to the area immediately anterior to the base.

The base is wide, and the very short midpiece attaches to one side. The sperm of this species is the smallest in total length of any studied.

Peromyscus eremicus

Figure 1G

SPECIMENS EXAMINED: Three. Arizona: Cochise County, Chiricahua Mountains (1). Texas: Brewster County (2).

The sperm head in this species resembles that of *P. californicus* in shape, although it is longer. The hook is of moderate length, extending backward about one-third the length of the head. The dorsal eave is prominent. The dense posterior region is confined to an area immediately anterior to the base of the head. The head base is relatively wide, and the midpiece attachment is highly eccentric. The midpiece is absolutely and proportionally longer than that of *P. californicus*, and the entire sperm is longer, although shorter than the mean of all species combined.

Peromyscus interparietalis

SPECIMEN EXAMINED: One. Mexico: Baja California, Salsipuedes Island.

The sperm head is shaped like that of *P. californicus*. The moderately long hook resembles that of *P. eremicus*. The dorsal eave is prominent. The dense posterior region is not sharply demarcated in stained specimens. The base of the head is broad, and the midpiece attachment is eccentric. No measurements are available for the sperm of this species.

SUBGENUS *OSGOODOMYS*

Peromyscus banderanus

Figure 1H

SPECIMENS EXAMINED: Three. Mexico: Guerrero, 0.5 miles west of Acahuiztla.

The sperm head is average in length, but narrow, being widest at about the midpoint. The hook has a very narrow base and is relatively long, in some cases extending nearly to the base of the head. The dorsal eave is prominent. In stained material, the entire sperm head is lightly stained, with no clearly differentiated darker posterior region. The base of the head is narrow, and the midpiece attachment varies from strongly eccentric to nearly central. Proportional lengths of the midpiece and tail are close to the average of all species.

SUBGENUS *PODOMYS**Peromyscus floridanus*

Figure 1I

SPECIMENS EXAMINED: Five. Florida: Alachua County, 7 miles west of Gainesville (1); Levy County, 15 miles southwest of Otter Creek (4).

The sperm head is close to average in width but slightly shorter than average in length. The hook is short, extending backward approximately one-third to one-half of the head length. The hook appears narrower and weaker than that in most species. The dorsal eave is not prominent. The heads of stained specimens were pale throughout, there being no distinct dense posterior region. The base is relatively narrow, but the midpiece attachment is strongly eccentric. The midpiece is relatively short and the tail proportionally longer than average.

SUBGENUS *ISTHMOMYS**Peromyscus flavidus*

SPECIMENS EXAMINED: Three. Panama: Los Santos.

The sperm head approximates the average in width, but is long, rounded in outline, tapering gradually to its apex. The hook is long and strongly recurved, extending back approximately one-half the length of the head. The dorsal eave is prominent. Stained heads are dark throughout with a darker line along the extreme base. Base of the head is narrow, and the midpiece attaches slightly off center. The midpiece is shorter and the tail longer than average in relative size.

Peromyscus pirrensis

Figure 1J

SPECIMENS EXAMINED: Two. Panama: Darién.

The sperm head is similar to that of *P. flavidus* in length and width. It also resembles that of *P. flavidus* in shape, although the hook is shorter. The dorsal eave is prominent. The dense posterior region is evident in stained material and occupies the extreme basal area of the head, except for an anterior extension on the side with the dorsal eave. The base of the head is narrow, and the midpiece attaches slightly to one side. The midpiece is relatively short and the tail proportionally long.

SUBGENUS *MEGADONTOMYS**Peromyscus thomasi*

Figure 1K

SPECIMENS EXAMINED: Two. Mexico: Guerrero, Puerto Chico, 63 km. by road southwest of Casa Verde.

The sperm head is slightly larger than average in length and width, and the hook originates broadly from the apex. The hook is long and strongly recurved, extending backward about one-half the length of the head. The dorsal cave is inconspicuous. The stained head is uniformly dark overall. The base of the head is narrow, and the midpiece attachment nearly central. The midpiece is actually longer than that of any other species but only slightly longer than average in relative size. The tail is proportionally short. Except for *P. megalops*, the sperm of this species has a greater total length than all others measured.

SUBGENUS *HABROMYS**Peromyscus lepturus*

Figure 1L, M

SPECIMENS EXAMINED: Six. Mexico: Oaxaca, south slopes of Cerro Pelon, 13 road miles northeast of Llano de las Flores (2): 12 miles north of Ixtlan de Juarez (4).

Two distinctly different types of sperm were observed in this species. The most abundant form was strikingly different from the hooked type typical of *Peromyscus* in other subgenera (fig. 1 M). The head is long and very broad. It lacks any trace of a hook and is symmetrical in outline. The head is generally pear-shaped, being broadest near the anterior end. Considerable individual variation is seen in the degree of expansion of the anterior portion. The narrow base of the head is smoothly rounded, rather than truncate as in other *Peromyscus*. In stained preparations, the anterior, swollen part of the head has a relatively clear acrosomal cap. The sperm head of *P. lepturus* is narrow in lateral aspect and nearly the same thickness as the midpiece. The head appears to be twisted, with a broad S-shaped outline in end view.

The midpiece attaches centrally and the difference in width of midpiece and base of the head is much less pronounced than in other *Peromyscus*. The midpiece is distinctly longer than average in actual measurement as well as in relative size, and the tail is proportionally very short compared with that in other subgenera.

In sperm preparations of two of the six specimens of *P. lepturus* examined,

spermatozoa of the characteristic hooked type were observed (fig. 1 L). The heads were relatively long and narrow. The hook was long and strongly recurved, and the dorsal eave was well developed. The midpiece attachment was slightly offset. When hooked sperm were initially observed on the slides of this species, we suspected contamination with material from other species. However, the fact that these variants were actually present in the cauda epididymis of *P. lepturus* along with the hookless form was verified by examination of wet mounts that were carefully prepared to eliminate any chance of contamination.

Unfortunately, epididymal sperm were sparse in the two specimens containing hooked sperm and thus it was impossible to obtain an accurate estimate of the relative frequency of the two sperm types. Counts of sperm in random fields of wet mount preparations yielded 28 (56 percent) hooked and 22 (44 percent) hookless types in one specimen and one hooked and two hookless forms in another specimen. Of the remaining four animals from which sperm were examined, one had relatively few sperm but all the 45 observed in random fields were hookless. Copious amounts of sperm were contained in preparations from the other specimens and only the hookless type was seen in careful scanning of the slides.

The limited data suggest the occurrence of individual and, possibly, geographic variation in the occurrence and relative frequency of the hooked sperm. The two mice containing both sperm types were from one of the two localities (south slopes of Cerro Pelon, 13 road miles northeast of Llano de las Flores) from which specimens were examined, and those with only the hookless types were from the other.

Measurements of the sperm of this species given in table 1 are of the typical hookless form.

Peromyscus lophurus

Figure 1N

SPECIMENS EXAMINED: Two. Guatemala: Department of Huehuetenango, Yayquich.

Although easily separable, the sperm of this species are obviously of the same type as the typical hookless form of *P. lepturus*. The sperm head of *P. lophurus* is relatively variable, widest through the middle, often having an irregular and asymmetrical outline. Some heads tend to have a broad diamond shape and some have a blunt process at the apex. The head is relatively longer and broader than that of any other species measured.

The midpiece attaches centrally. As in *P. lepturus* it is actually and relatively very long. The tail is the shortest of any species studied.

Unlike the case of *P. lepturus*, no hooked type sperm were observed in preparations containing copious sperm, despite careful search.

DISCUSSION

This study indicates that, although the differences are generally subtle, the spermatozoa of each species of *Peromyscus* tend to be characterized by a particular combination of features, including shape and staining properties of the head and absolute and relative size of the head, midpiece, and tail components. Because of small sample sizes the results should be regarded as provisional. Furthermore, it must be emphasized that only the more conspicuous differences were likely to be demonstrated by the methods employed and that more detailed comparisons utilizing histochemical techniques or fluorescence or electron microscopy would most likely reveal additional, and perhaps more fundamental, patterns of variation.

Although there are numerous interspecific differences among *Peromyscus* sperm, only two major patterns exist within the genus: the relatively large-headed, hookless form found in *P. lepturus* and *P. lophurus* of the subgenus *Habromys* and the hooked type possessed by all other species examined and which is characteristic of many other murids. In the absence of a hook and the globose shape of the sperm head, the sperm of *Habromys* approaches that of *Ochrotomys* (Hirth, 1960), which was formerly regarded as a subgenus of *Peromyscus*.

The existence of both sperm types in some individuals of *P. lepturus* is a puzzling and apparently unusual condition. Presumably this dimorphism reflects differences in genetic factors having an influence on sperm morphology. Braden (1960) reviewed several cases of known genetic effects on gametic phenotypes in other mammals. The occurrence of two such sharply distinct variants with no intermediate forms suggests that the underlying genetic mechanism may be relatively simple. However, this interpretation is difficult to reconcile with the usual constancy of sperm shape within a species. As the hooked-type sperm is a more specialized condition than the simpler hookless form, the sperm of *Habromys* may be regarded as representing either the retention of a primitive form or a secondary development of a simpler type from a hooked form. In view of the prevalence of hooked sperm among other *Peromyscus* and murids in general (Friend, 1936; Bishop and Austin, 1957), the second suggestion appears more probable. The relatively specialized male genital tract of this subgenus also supports this assumption (Linzey and Layne, 1969). Thus, the dimorphic sperm in some *P. lepturus* can be viewed as either an early stage

in a transition from a less specialized sperm to the more complex hooked type or, which seems more likely, a late stage in the transition from a hooked to hookless type. A third possibility is that the sperm dimorphism is simply the result of some unusual genetic or developmental fluke and has no direct phylogenetic significance.

Among *Peromyscus* with hooked sperm, there are no definite patterns of variation in qualitative or quantitative characteristics of the sperm that result in any obvious clustering of species. Therefore, other than *Habromys* the subgeneric groups that are well defined on the basis of anatomy of the male genital tract (Hooper, 1958; Linzey and Layne, 1969) and other structures are not obviously reflected in sperm variation as revealed by the present study. The same is true at the species group level within the subgenus *Peromyscus*. Although in some instances, as in the case of *P. maniculatus* and *P. polionotus* of the *maniculatus* group, closely related species show similarities in sperm morphology, others, for example *P. leucopus* and *P. gossypinus* of the *leucopus* species group, do not.

From a taxonomic standpoint, therefore, sperm morphology of *Peromyscus* at the level investigated in this study is of relatively limited usefulness in assessing relationships between species within the genus. This is in contrast to the situation found in the structure of the male genital tract. Although *Habromys* differs drastically from other subgenera in sperm type, it is no more divergent in the morphology of the male genital tract than such subgenera as *Podomys*, *Osgoodomys*, or *Isthmomys*. On this basis, therefore, sperm morphology appears to be more conservative than the structure of the genital tract itself.

In the case of *Habromys*, the markedly different sperm coupled with the distinctive features of the genital apparatus adds further support to our earlier suggestion (Linzey and Layne, 1968) that the genus *Peromyscus* as now constituted is probably not a natural assemblage. The reproductive features distinguishing *Habromys* from other subgenera of *Peromyscus* are fully equivalent to those which supported the earlier removal of *Ochrotomys nuttalli* from the genus *Peromyscus* (Hooper, 1958; Hirth, 1960; Arata, 1964). In addition, given the wide distribution of the hooked sperm type among remotely related murids, the presence of this form in *Peromyscus*, which otherwise differ in genital anatomy or other characteristics, does not necessarily indicate fundamental relationship.

Forman (1968) found that although large bats tend to have large spermatozoa, there appeared to be no consistent correlation between body size and sperm size. Our data for *Peromyscus* lead to a similar conclusion. Although the largest species examined, *P. thomasi* and *P. megalops*, also had the largest spermatozoa (mean total length, 90.0 and 93.9 microns,

respectively), another species with large body size, *P. californicus*, had the smallest sperm encountered (mean, 72.0 microns).

SUMMARY

Spermatozoa of 27 species of *Peromyscus* representing all seven subgenera were studied. Means and ranges (in microns) of measurements of various sperm components of 25 species were: head length, 5.5 (5.1–6.2); head width, excluding hook if present, 3.0 (2.4–4.9); midpiece length, 17.5 (15.2–21.4); tail length, 56.0 (49.3–69.3). Although differences are generally subtle, interspecific variation occurred in head shape and absolute and relative dimensions of head, midpiece, and tail. Two major types of sperm occurred among the species examined. Members of the subgenus *Habromys* possessed sperm with relatively large heads without a hook, whereas all other species had sperm with a head hook typical of many murids. Some individuals of *P. lepturus* in the subgenus *Habromys* had both hookless and hooked sperm, with the latter type being scarcer. It seems more likely that the hookless sperm of *Habromys* was secondarily derived from a hooked type rather than representing retention of a primitive pattern. Although specific variation is evident in the sperm of the remaining species, there are no consistent patterns of variation corresponding either to subgenera or the currently recognized species groups within the subgenus *Peromyscus*. The distinctive sperm of the subgenus *Habromys* coupled with well-marked genital and other characters strengthens the suspicion that the genus *Peromyscus* as presently constituted is not a natural assemblage. Possession of the basically similar hooked type sperm by all remaining subgenera, some of which are as divergent as *Habromys* in the structure of the male tract, does not necessarily indicate special affinity among these groups, as this sperm type is common to many murid rodents. Although the two largest species studied also had the largest spermatozoa, there is no clear-cut correlation between body size and sperm size in the series as a whole.

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