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MALE ACCESSORY GLANDS AND THE EVOLUTION  
OF COPULATORY PLUGS IN RODENTS

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INTRODUCTION

Many rodents, particularly of the suborders Caviomorpha and Myomorpha, possess unusually well-developed and elaborate male accessory reproductive glands. Murids (*sensu* Hooper and Musser, 1964), especially, display the largest and most complex glandular complements: three to four distinct and conspicuous prostatic lobes may be present, vesicular glands (=seminal vesicles) are very large, and bulbo-urethrales and ampullaries are prominent (Price and Williams-Ashman, 1961; Arata, 1964). The problem this remarkable glandular development poses the evolutionary biologist was summarized succinctly by Asdell (1966:2):

"The seminal vesicles and bulbo-urethral glands are absent in Carnivora and Cetacea, while they are highly developed in Rodentia. In the latter order, too, the prostate has undergone a high degree of differentiation. One wonders about these adaptations, if they may be so named. Carnivores do without some of the accessory organs while in the rodents they are highly developed. What biological significance have they? Their removal in some of the species in which they are well developed has little effect upon fertility."

Less widely known is that considerable variation in these organs exists among rodents. Price (1963:1) noted that "Variability in the glands represented exists even between families within orders [of mammals]", yet Arata (1964) found a great diversity of glandular types among some North American murid genera, and Linzey and Layne (1969) demonstrated considerable differences in accessory gland complements among species of the genus *Peromyscus*.

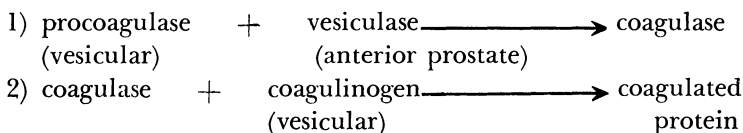
In the course of a study of the comparative gross morphology of male accessory glands in neotropical murids (Voss and Linzey, in preparation), I became interested in the selective pressures responsible for the complexity and variability of this organ system. Here I review relevant aspects of the large and diverse literature on rodent reproductive physiology, anatomy, behavior and ecology, and indicate which adaptive considerations seem likely to have been most important in the evolution of rodent accessory glands and their secretory products.

#### MALE ACCESSORY GLAND SECRETIONS

The only function of mammalian male accessory glands is secretion of the seminal plasma (Price and Williams-Ashman, 1961), and yet a bewildering variety of organic and inorganic substances is produced. To list all of these and their hypothesized roles in fertilization is beyond the scope of this paper; excellent reviews are provided by Barnes (1972), Mann (1964, 1974) and Price and Williams-Ashman (1961). Little agreement exists as to the significance of any chemical present in semen. As noted by Asdell (1966, see above) and demonstrated by Greenstein and Hart (1964), removal of some glands has no detectable effect on fertility or fecundity. None of the known products of mammalian accessory glands appear necessary for fertilization if artificial measures are taken to ensure contact between sperm and egg (Barnes, 1972; Mann, 1964), so capacitation of spermatozoa seems ruled out. Functions most commonly assigned the many components of seminal plasma are listed below; all are subsumed under the universally accepted primary role of this fluid, that of providing a mechanically and physiologically optimal medium for sperm delivery. Substances present in mammalian seminal plasma may (1) provide nutrients for the spermatozoa, (2) adjust the reduction-oxidation potential of the semen, (3) maintain osmotic balance in the semen, (4) maintain proper pH in the semen, or (5) stimulate smooth muscle contractions of the uterus (Mann, 1964;

Bishop, 1961). These functions, however, are of such general importance that none may be considered unique to rodents, nor expected to vary appreciably between related species. Indeed, substances usually identified with these roles are of widespread occurrence in the semens of other mammalian orders, and their secretion seems thus unlikely to have been responsible for the elaboration of rodent accessory gland complements. This is not to say that rodents may not accomplish these ends differently or more efficiently than other mammals, but only that the degree of development of glands and the diversity of glandular types in the order imply the existence of additional seminal functions, more or less peculiar to rodents, which vary between some species and are of substantial importance for successful reproduction. The only known function of rodent accessory gland secretions that satisfies the above criteria is the formation of a hard plug that remains in the female tract after copulation.

So-called copulatory plugs do not at first appear restricted to rodents, but have been reported also in some bats, insectivores, primates and marsupials (for an early review see Engle, 1926b). These vaginal occlusions differ from rodent plugs in many important details, however. Rodent copulatory plugs are typically hard, rubbery or waxy in consistency, and are the exclusive product of male secretions. Specifically, the rodent plug forms as the result of two denaturation reactions between proteins secreted by the enlarged vesiculars and anterior prostates (Walker, 1910; Speyer, 1959):



By contrast, the vaginal plugs of some vespertilionid bats consist of cornified epithelial cells of the upper vagina shed after mating (data summarized in Eadie, 1948; Kitchener, 1975), while the plug of *Rhinolophus* (Rhinolophidae) is formed from the coagulated secretions of male urethral glands (Racey, 1975). The gelatinous plug of *Didelphis* results from coagulation of vaginal secretions by the ejaculate (Hartman, 1924), and the paste-like plugs of some insectivores derive from secretions of prostates of uncertain homology and the bulbo-urethrales (Eadie, 1948). Coagula found in the ejaculates of some primates may be produced by homologues of rodent vesiculars and anterior prostates (Von Wagenen, 1936), but appear to disintegrate considerably *in vivo* (Blandau, 1973; Tinklepaugh, 1930), which is not a characteristic of rodent plugs.

Copulatory plugs are common and widespread in rodents; rodent species listed as plug-secreting by Asdell (1964) include representatives of nine families in four suborders. That very similar reactants secreted by apparently homologous glands account for plug formation in guinea pigs (Caviidae, Caviomorpha) and domestic rats (Muridae, Myomorpha) (Mann, 1964; Walker, 1910) argues the early acquisition of this biochemical process in rodent phylogeny. Only a handful of murids, to be discussed in more detail below, are known not to secrete copulatory plugs. These species possess reduced or modified glandular complements (Hartung and Dewsbury, 1978), an observation corroborating the hypothesis that plug secretion is an important function of rodent accessory glands.

#### COPULATORY PLUGS IN RODENTS: THE HYPOTHESES

The observed properties of rodent copulatory plugs (below) and knowledge of the biochemical reactants that produce them indicate that the plug is an evolved adaptation and not a fortuitous effect of normal reproductive-physiological processes — but an adaptation for what? Five functions of rodent copulatory plugs have been suggested in the literature. Plugs may (1) permit a gradual release of spermatozoa within the female tract as they disintegrate (Asdell, 1946), (2) prevent leakage of spermatozoa from the vagina (Leuckart, 1847), (3) induce pseudopregnancy in the female (Long, 1919), (4) transport sperm through the cervix (Blandau, 1945), or (5) prevent subsequent insemination of females by other males (Martan and Shepherd, 1976). These hypotheses are considered individually below.

**SPERM STORAGE** — Asdell (1946:31) believed the copulatory plug to be “. . . conducive to fertilization as it permits a gradual release of spermatozoa as it disintegrates.” Asdell presumably envisioned a sperm-storage role for plugs, advantageous when the time of ovulation is uncertain; inert spermatozoa are longer-lived than free-swimming sperm in the female tract (Asdell, 1946; Bishop, 1961). Credence is lent this hypothesis by the observation that, while many rodents are spontaneous ovulators in which periods of female receptivity (heats) are synchronized with the release of ova (Conaway, 1971), the onset of heat and of ovulation do not correspond exactly, and the resulting hiatus may be of appreciable length. The few relevant data are summarized in Table 1. As can be seen, ovulation does not coincide with the onset of female receptivity in the four species for which such information is available, but follows after

REPRODUCTIVE CHARACTERISTICS OF FOUR SPECIES OF RODENTS.

TABLE 1.

See text for discussion.

species	length of heat	time from the onset of heat to ovulation	fertilization life of:		tenure of the copulatory plug in the female tract	sources
			ovum	sperm (in female tract)		
<i>Mesocricetus auratus</i>	13-26 hours	8-9 hours	6-13 hours	7 hours	extruded "rather soon" after copulation	Asdell, 1964 Blandau, 1969 Kent, 1968 Bentley and Soderwall, 1939
<i>Rattus norvegicus</i>	13.7 ± 4.5 hours	8-11 hours	10-15 hours average: 12-13 hours	14 hours	12-24+ hours	Asdell, 1964 Long and Evans, 1922 Lisk, 1969 Soderwall and Blandau, 1941
<i>Mus musculus</i>	about 12 hours	2-3 hours (variable)	15 hours	6 hours	18 hours - two days	Asdell, 1964 Blandau, 1969 Snell, 1956 Parkes, 1926 Merton, 1939
<i>Cavia porcellus</i>	6-11 hours in 90% of all cases	10 hours	8-26 hours	20-22 hours	9.5-18 hours	Martan and Shepherd, 1976 Asdell, 1964 Stockard and Papanicolaou, 1919 Soderwall and Young, 1940

intervals of two to eleven hours. In three species, however, these intervals are exceeded by the fertile life of spermatozoa in the female tract; of the four, only a male *Mesocricetus* would thus appear to be incapable of fertilizing ova if copulation occurred during the first hour or two of heat. Copulatory plugs are also widespread in induced ovulators of the genus *Microtus* which generally shed ova about ten hours after copulation (Cross, 1972; Richmond and Conaway, 1969a), but the fertile lives of spermatozoa in the female tracts of these species are unknown.

The data in Table 1 are not entirely consistent with Asdell's (1946) hypothesis, but it could be argued that since sperm viability decreases with time, the gradual release of fresh sperm from a disintegrating plug could still increase male fertility, particularly if matings frequently occur at the onset of heat. Observations on the consistency of the plug in the female tract, however, lend no support to this interpretation, nor, in fact, to any version of the sperm-storage hypothesis. Descriptions of copulatory plugs consistently omit any mention of the disintegration to which Asdell (1946) alludes; eventual dislodgement is effected, not by any deterioration of the plug itself, but by an involved process of delamination of outer, cornified layers of the vaginal epithelium (Engle, 1926a; Long and Evans, 1922; Parkes, 1926; Stockard and Papanicolaou, 1919). Asdell (1964) cites Parkes (1926) as reporting plug disintegration in *Rattus norvegicus*; no such statement is, in fact, made by Parkes, and I can only conclude that Asdell is in error here. Koren *et al.* (1974, 1975) discovered that a collagen-like peptidase (CLP) secreted by the spermatozoa of *Homo*, *Bos* and *Rattus* is capable of degrading, *in vitro*, proteins secreted by the rat vesicular gland that normally interact with anterior-prostatic vesiculase to form the copulatory plug. Vesicular proteins incubated with CLP did not coagulate when vesiculase was subsequently added. Koren *et al.* (1975: 495) concluded ". . . it seems reasonable to suppose that this enzyme [CLP] may assist spermatozoa to escape from the plug *post coitum*." These authors did not show, however, that spermatozoa really are released from copulatory plugs, nor that CLP is capable of lysing the plug coagulum once it is formed. Martan and Shepherd (1976) placed fresh copulatory plugs in the vaginas of receptive but unmated female guinea pigs; none of the females conceived, and these authors concluded that sperm storage is an unlikely function of the plug in this spontaneously ovulating species. Live spermatozoa trapped in the plug matrix appear never to be freed (Blandau, 1969, 1973; Engle, 1926a), and their presence

there seems best attributed to a fortuitous mixing of glandular secretions with part of a fluid, pre-plug sperm packet. The presence of such a sperm-containing, non-coagulating fraction of the ejaculate is suggested by the abundance of spermatozoa in uterine fluids 60 seconds after copulation in domestic rats (Hartman and Ball, 1930), and by direct observations of the ejaculates of domestic rats (Long and Evans, 1922) and guinea pigs (Engle, 1926a; Walton, 1960).

SPERM LEAKAGE — Leuckart (1847; cited in Stockard and Papanicolaou, 1919) was apparently the first to suggest that rodent copulatory plugs function in preventing leakage of sperm from the vagina, an hypothesis which has subsequently gained wide acceptance (e.g. Long and Evans, 1922; Mann and Lutwak-Mann, 1951). It is curious, however, that no direct evidence apparently exists to indicate that such leakage actually obtains for rodents. Prevention of sperm leakage is an unlikely role for the plugs of *Mus musculus*, *Rattus norvegicus* and *Mesocricetus auratus* since in these species the spermatozoa are not deposited in the vagina at ejaculation, but in the uterus, where they are retained by a sphincter-like action of the constricted cervix (Blandau, 1973). Blandau and Odor (1949) found few sperm in vaginal washings of mated female domestic rats *versus*  $31-85 \times 10^6$  spermatozoa retained by the cervix in the uterine cornua. Similarly, dye injected into the uteri of domestic rats does not appear in the vagina while the cervix remains constricted during heat (Blandau, 1945). Rugh (1968:46) asserts of *Mus musculus* that "Some semen passes out through the cervix, but since the vaginal plug may persist for several days, more remains in the uterus." Since Rugh provides no procedural details, it is unclear whether an actual observation is being reported or conventional wisdom repeated. Martan and Shepherd (1976) removed the copulatory plugs from six female guinea pigs immediately after copulation. The cervix is not constricted in this species, but remains open throughout heat. Five of the six females conceived normal-sized litters. This result in a species whose anatomy might lead one to expect sperm leakage to be important lends little support to Leuckart's (1847) hypothesis.

Land and McGill (1967) reported a reduction in fertility of female domestic mice when copulatory plugs were removed immediately after copulation, but did not comment on what factors might have been responsible for the effect. This could be taken as evidence supporting the sperm leakage hypothesis, but sperm loss was not documented. These same authors noted that plug removal was difficult, and the trauma of removing plugs introduces additional variables

known to reduce fertility in other murids. Adler and Zoloth (1970) found that direct stimulation of the cervix soon after copulation reduces uterine sperm counts and numbers of implanted zygotes in *Rattus norvegicus*; such stimulation must attend plug removal, and may account for Land and McGill's (1967) results. A control group of mice in which plugs are comparably agitated but not removed might yield more readily interpretable data if these experiments are repeated in the future.

I am not aware of any morphological evidence to suggest that sperm leakage should be any more of a problem for rodents than for other mammals which do not secrete plugs. Sperm leakage is not an easy phenomenon to observe, nor, having been observed, can it easily be said that a selective advantage necessarily accrues to its prevention; the gametic profligacy of male mammals is such that the loss of a few million sperm may have no real effect on fertility. Little can be said for an evolutionary hypothesis which assumes a selective pressure that has never been shown to exist.

PSEUDOPREGNANCY INDUCTION — Following ovulation in mammals, the ruptured Graafian follicle forms the corpus luteum, an endocrine organ which secretes progesterone, thereby maintaining the uterine linings in a highly vascularized condition and inhibiting further ovulations. If fertilization occurs, luteal activity is prolonged beyond its normal length in the nonpregnant cycle by hormones secreted by developing fetal tissues. If fertilization does not occur, the corpus luteum regresses, progesterone is no longer secreted, and uterine linings are resorbed. In most mammals, progesterone secretion by the corpus luteum follows ovulation automatically, but in some genera of spontaneously ovulating murid rodents corpora lutea remain non-functional unless activated by a poorly-understood neuro-endocrine reflex triggered by copulation. Activation of the corpus luteum and initiation of the luteal phase of the female reproductive cycle are critical for successful pregnancy. If, however, the mating was sterile, induced progesterone secretion continues nonetheless, the next ovulation is delayed (for up to two weeks depending on the species), and the female is said to be pseudopregnant. The unfortunate use of 'pseudopregnant' connotes a qualitative distinction, not recognized by most workers in mammalian endocrinology, between this phenomenon and the normal luteal phase of pregnancy. Because pseudopregnancy is easily observed in the laboratory, it has been intensively studied through the use of vasectomized males, chiefly in *Rattus norvegicus* and *Mus musculus*, in order to determine which of the



stimuli accompanying insemination induces luteal activity.

To return to copulatory plugs: Ball (1934) observed that female *Rattus norvegicus* mated with vasectomized males rarely became pseudopregnant if copulatory plugs were not deposited at ejaculation, and that the incidence of pseudopregnancy increased markedly when plugs were secreted; she concluded that plugs provide a necessary mechanical stimulus for induction of pseudopregnancy. Long (1919) had previously speculated that copulatory plugs might function in this way. In a series of well-controlled experiments, however, Adler (1969) showed that plug formation is neither necessary nor sufficient for luteal activation in rats. This author found that the number of preejaculatory intromissions was the critical factor, and suggested that the discrepancy between his results and Ball's (1934) might be due to Ball's failure to accurately quantify the number of intromissions received by female rats in which plugs were not deposited. Attempts to duplicate Ball's results for *Mus musculus* have also failed (McGill *et al.*, 1968). Male *Mesocricetus auratus* from which plug-secreting glands have been surgically removed can successfully impregnate females (Weinerth *et al.*, 1961), so luteal activation does not appear to require plug formation in this species either. Furthermore, secretion of copulatory plugs is widespread in caviomorph rodents which spontaneously form fully active, functional corpora lutea after ovulation (Wier, 1974; Wier and Rowlands, 1974).

Pseudopregnancy induction may only be admitted as a possible ancestral function of the plug if it can be shown that the formation, at ovulation, of corpora lutea requiring copulatory activation is an earlier, or at least equally early, acquisition in rodent phylogeny than plug secretion. Conaway (1971:241), however, regarded this type of female reproductive pattern as "highly specialized", and observed that it is restricted in taxonomic distribution to a few genera of murid rodents only. These observations do not support recognition of an induced luteal phase as the primitive condition for plug-secreting rodents. That the plug may have the effect of inducing pseudopregnancy in some species is not here contested, and will be discussed in more detail below. The phenomenon seems uncommon in any case, having been demonstrated only once for domestic rats.

**SPERM TRANSPORT** — The cervixes of some murid rodents are tightly constricted during heat, and effectively prevent leakage of uterine fluids during this period (see above). Sperm suspended in appropriate solutions and introduced into the vaginas of receptive female *Rattus norvegicus* will not enter the uterus, nor will the ejaculates of male

rats from which vesiculars and anterior prostates have been surgically removed (Blandau, 1945). These and other observations led Blandau (1945) to suggest that the copulatory plug functions to transport sperm through the murid cervix. He believed that the stimulus provided by plug formation at ejaculation may induce female orgasm, during which the extreme tonicity of the cervix is momentarily relaxed and sperm entry is permitted. Blandau's (1945) reconstruction of the events responsible for sperm transport in *Rattus* appears logical and consistent with his data, but I question the generality of the effect. A tightly constricted cervix has been described for seven genera of rodents only (Blandau, 1973; Hartung, 1976), all of them murids, and would thus not seem to be taxonomically widespread (because the cervical morphologies of only a few rodents are known, however, this conclusion may be premature). The effect, too, has been observed for only one laboratory species; that male domestic mice and hamsters are not rendered sterile by removal of plug-secreting glands (McGill *et al.*, 1968; Weinerth *et al.*, 1961), indicates that plug formation is not critical for sperm transport in all murids. Plugs are also well developed in guinea pigs in which the cervix is known to remain open throughout heat; sperm suspensions artificially placed in the vaginas of receptive females will enter the uterus unaided, and effect fertilization (Blandau and Young, 1939).

Removal of either anterior prostates or vesiculars may reduce the percentage of fertile matings in male *Mus musculus* and *Cavia porcellus* (Engle, 1926a; McGill *et al.*, 1968), but it does not follow that this is due to lack of plug formation *per se*. Secretions of these glands comprise a large proportion of the ejaculate, and their absence must necessarily reduce the force with which the sperm-containing liquid fraction is evidently propelled through the cervix (Blandau, 1945). Complete removal of anterior prostates, the secretions of which are necessary for plug formation, does not reduce either litter size or the percentage of fertile matings in *Rattus norvegicus* (Greenstein and Hart, 1964). This suggests that the sterility Blandau (1945) induced in male rats by removing both vesiculars and anterior prostates may not have been due to lack of plug formation, but instead to a reduced volume of the whole ejaculate. Because male-female responses associated with ejaculation are tightly coevolved, removal of organs that normally participate in the mating process may produce secondary effects that have little to do with the organ's real function.

CHASTITY ENFORCEMENT — If it were found that copulatory plugs result from interactions of specialized male *and* female secretions,

then it could be said with confidence that some benefit must accrue to the female from plug formation. As it is, the fact that rodent plugs are the products of exclusively male secretions is at least consistent with a hypothesized plug function of advantage only to the male. One such function, of benefit solely to the male, is chastity enforcement: prevention of insemination of the female from subsequent matings with other males.

A chastity-enforcing function for male-deposited oviductal or vaginal occlusions is not a novel concept. Such a role for plugs was proposed by Parker (1970) for many insects, by Devine (1975, 1977) for some colubrid snakes, and by Racey (1975) for rhinolophid bats. A series of elegant experiments by Martan and Shepherd (1976) led these authors to conclude that the plug of guinea pigs may also function in this way. Testing the validity of this hypothesis as a general explanation for rodent copulatory plugs requires answers to at least two questions: (1) Do the mating systems of rodents provide an appropriate selective context for the evolution and maintenance of chastity-enforcing plugs? (2) Are the general properties of rodent plugs consistent with such a hypothesized role?

### 1. The Selective Context

Since most rodents are nocturnal, little is known of their social behavior. The great majority appear to live singly as adults, and permanent pair-bonding is extremely rare (Eisenberg, 1966). Although Kleiman (1977) listed 28 species of rodents judged monogamous by her, the criteria she employed seem to me poor predictors of mating exclusivity. In fact, females of *Peromyscus maniculatus*, listed by Kleiman (1977) as monogamous, show high proportions of multiply-inseminated litters in wild populations (Birdsall and Nash, 1973; Merritt and Wu, 1975). Multiple matings have also been observed under natural or seminatural conditions for *Microcavia australis*, *Rattus norvegicus* and *R. rattus*, and in captivity for *Mus musculus*, *Peromyscus leucopus*, *Clethrionomys glareolus*, and *Cavia porcellus* (Calhoun, 1963; Clarke *et al.*, 1970; Dewsbury, 1976; Rood, 1970; Rugh, 1968; Martan and Shepherd, 1976). These data, together with the general absence of pair-bonding noted above, suggest that widespread promiscuity may exist among female rodents in nature. Why a female rodent should 'wish' to mate repeatedly is an interesting question, but is irrelevant to the chastity-enforcement hypothesis: as long as multiple matings *do* occur, for whatever reason, it is almost

certain to be in the male's interests to see that later, competing copulations are ineffective.

## 2. Properties of Rodent Plugs

As emphasized by many authors (e.g., Blandau, 1973, and references therein), rodent copulatory plugs are typically hard and firm in contrast to the seminal coagulums of other mammals. Parkes (1926:155) noted that vaginas dissected from female *Mus musculus* with the plug *in situ* were difficult to section as the plug “. . . becomes extremely hard and practically uncuttable.” Long and Evans (1922:73) observed that the plug of *Rattus norvegicus* “. . . adheres so tightly to the cornified layer of the vaginal mucosa that it is not easily distinguished in section.” Similarly, Rugh (1968:46) noted, “It [the plug of *Mus musculus*] hardens to such a degree that mechanical removal can injure the vaginal mucosa and the uterine ligaments.” Descriptive literature summarized in Table 2 suggests that the passages quoted above do not describe features unique to plugs of laboratory species, nor to those of a few genera only. These properties of rodent plugs seem to me entirely consistent with, and appropriate to, an hypothesized role in chastity-enforcement. The appearance in vaginas of some rodent species of solid masses that are the products of female estrous discharges, not male secretions (Meyer and Meyer, 1944; Kent, 1968), enjoins caution in accepting vague or casual references to ‘plugs’ by authors not otherwise concerned with this phenomenon. Accordingly, I have omitted from Table 2 descriptions of plugs recorded in contexts that render their male origins suspect, e.g., the appearance of plugs in female *Dipodomys spectabilis* “. . . about 12 to 15 hours after copulation” (Butterworth, 1961:414).

It can also be seen from Table 2 that copulatory plugs frequently remain in the female tract for extended periods ranging, where actual times are provided by the writers, from 9½ hours to two days. In *Mus musculus* the normal tenure of copulatory plugs in the vagina exceeds in length both heat and the interval from onset of heat to the end of the fertilizable life of the ovum (Table 1). In *Rattus norvegicus* and *Cavia porcellus* plug tenure closely approximates the normal length of heat (Table 1). In all three cases, plugs remain in the female fully (or very nearly) as long as one would expect of a chastity-enforcing mechanism. Observations of rapid (but unquantified) plug loss are implied by some authors (e.g., for *Lagidium peruanum* and *Clethrionomys glareolus*, Table 2), however, and occa-

TABLE 2.

DESCRIPTIONS OF COPULATORY PLUGS OF 19 SPECIES OF RODENTS. Sources: (1) Long and Evans, 1922; (2) Parkes, 1926; (3) Hartung, 1976; (4) Brambell and Hall, 1939; (5) Richmond and Conaway, 1969b; (6) Hamilton, 1941; (7) Benton, 1955; (8) Brambell and Rowlands, 1936; (9) Stockard and Papinacolaou, 1919; (10) Martan and Shepherd, 1976; (11) Asdell, 1964; (12) Pearson, 1949; (13) Weir, 1971; (14) Deanesly and Parkes, 1933.

Species	Tenure of plug within the female tract	Description of plug
<b>MYOMORPHA</b>		
<i>Rattus norvegicus</i>	12-24 hours	"[plugs] carry with them various portions of the cornified layer of the vagina, to which the plug is adhering tightly, the cornified layers being in the act of dehiscence at this time" (1:72)
<i>Mus musculus</i>	18-24 hours	"may persist for 36 hours or two days"; "Evacuated plugs coated with cornified epithelium"; "seals the vagina even more completely than in the case of the rat" (2:154)
<i>Peromyscus leucopus</i>	---	"hard but rubbery"; "adheres to the vaginal wall making it difficult to remove" (3:22)
<i>P. gossypinus</i> , <i>P. maniculatus</i>	---	"hard and brittle"; "stuck tightly to the vaginal wall" (3:22)
<i>P. eremicus</i>	---	"soft" (3:23)
<i>Mesocricetus auratus</i>	---	"hard and rubbery"; "adheres to the vaginal wall" (3:24)
<i>Meriones tristrami</i>	---	"hard" (4:136)
<i>Microtus agrestis</i>	---	"hard rubbery"; "adheres tightly to the vaginal walls" (3:23)
<i>M. californicus</i>	---	"when crushed, has a fibrous appearance" (5:86)
<i>M. ochrogaster</i>	24 hours	"waxy" (6:8)
<i>M. pennsylvanicus</i>	"about two days"	"viscous mass" (7:56)
<i>M. pinetorum</i>	"probably persists for several days"	"hard" (8:89)
<i>Clethrionomys glareolus</i>	"short time"	
<b>CAVIOMORPHA</b>		
<i>Cavia porcellus</i>	9.5-18 hours	"rigid"; "surrounded or enclosed by a mass of flat epithelial cells, apparently derived from the vaginal wall" (9:229, 233; 10)
<i>Chinchilla laniger</i>	---	"shed entire, surrounded by the cornified layer of the vaginal wall" (11:404)

Species	Tenure of plug within the female tract	Description of plug
<i>Lagidium peruanum</i>	"only a short time"	"hard waxy"; "a rather tough film of cornified vaginal epithelium envelops the plug when it is expelled"; "[plugs] expand the vagina considerably while they are in situ" (12:149-150)
<i>Lagostomus maximus</i>	---	"gelatinous"; "whole plug covered by sloughed cornified tissue" (13:362, caption to figure 2)
SCIUROMORPHA	---	
<i>Sciurus carolinensis</i>		"firm jelly-like mass" (14:55)

sional or even frequent plug dislodgement accompanying subsequent copulations or intromissions has been documented for a few species. Such observations do not, at first, seem consistent with the hypothesis that plugs enforce female chastity, and so merit further discussion.

Mosig and Dewsbury (1970) and Miligan (1975) have observed that, when additional copulations or intromissions follow very soon after plug deposition by the same male, dislodgement of a plug may occur. Neither of these authors, however, ascertained whether or not plugs remain in the female after the *last* copulation between pairs. Speaking to this latter point, Lisk (1969:315) observed for *Rattus norvegicus*: ". . . the copulatory plug does not fall out within a few hours of copulation unless dislodged by succeeding intromissions [following plug desposition by only a few minutes in his tests], but in over 50% of our observations tends to remain for 24 hours or longer in the female's vagina." That deposited plugs may go unnoticed by investigators was further suggested by the same author: ". . . in over 90% of the ejaculations the plug was lodged too deep to be visible externally" (Lisk, 1969:315). Without very careful observation I do not see how it can be determined whether the "dislodged" plug was the one first deposited (a failure), or that formed from subsequent ejaculations because of an inability to fully penetrate the already occluded vagina, in which case the first plug was a success. Where females have actually been killed and dissected, the normal situation appears to be nondislodgement or plug accumulation. In four murid species for which such data are available, incidences of complete plug dislodgement range from 0 to 25% only; incidences of complete retention or multiple plug accumulation range from 72 to 100% (Hartung, 1976). This author noted further that dislodgement may be an experimental artifact, and that plugs deposited at ejaculation

require about 15 minutes to harden completely, after which they are difficult to remove. The normal interval, in nature, between competing copulations is, of course, unknown. That plugs do not fully harden sooner may well be due to biochemical constraints on the rate at which the necessary reactions can occur: no adaptation is perfect!

It could be argued that (1) plugs which can be dislodged at all while females are still capable of being fertilized do not display sufficient tenacity to justify recognition of a chastity-enforcing mechanism, and (2) in the few documented cases of plug dislodgement it is the male which deposited the plug in the first place that effects its dislodgement by repeated intromissions, and that such behavior would not seem to indicate any great concern on the part of the male with the continued presence of his plug in the female.

To the first objection I would note that plugs appear to stick very tenaciously indeed *to the vaginal mucosa*, the only female tissue they contact, a fact widely attested by authors who have described the surfaces of shed plugs. Such plugs are, without reported exception, covered with adherent sheets of epithelium (Table 2). Delamination of these cell layers is effected by large numbers of leucocytic cells that invade the vagina shortly after copulation (Stockard and Papanicolaou, 1919; Engle, 1925). Long and Evans (1922:73) speculated that dehiscence of this epithelium may be “. . . a means of insuring loss of the plug after it has performed its function [prevention of sperm leakage].” If the massive vaginal exfoliation of female rodents is to be regarded as an evolved mechanism associated with plugs, and if the chastity enforcement hypothesis is correct, I suggest instead that shedding of vaginal linings might more appropriately be regarded as a means to ensure loss of plugs *before* they have performed their function, since that function can hardly be construed as beneficial to females. The point I wish to make, in brief, is that plug loss does not appear attributable to any property of the plug *per se*, and thus cannot be interpreted to imply that plugs do not display, within the mechanical constraints imposed by the female tract, sufficient tenacity to be regarded as evolved chastity-enforcing mechanisms.

Regarding the second objection, Dewsbury (1972, 1975) and others have found that repeated copulations may function in inducing pseudopregnancy in female murid rodents, thereby preventing resorption of uterine linings and the resultant loss of fertilized ova. It is this behavior that occasionally effects plug dislodgement (Miligan,

1975). Thus, disadvantages, in the context of chastity-enforcement, associated with disturbing an as-yet-not-fully-hardened plug by repeated copulations may be more than offset by an increased probability of inducing successful pregnancy. As mentioned above, however, there exists no evidence that a copulatory plug is not regularly retained in the female tract after the last copulation between a given pair. I see no reason why, in the rapid course of several copulatory bouts with the same female, a male should exhibit concern for the fates of deposited plugs so long as one is implanted at the last ejaculation and then left to harden fully. It does seem inefficient for a male to deposit supposedly chastity-enforcing plugs after each mating if he is assured of copulating again with the same female in a very short time. However, in nature males may never have this assurance. Copulating pairs may be interrupted by predators or rival males, or females may desert after an unpredictable number of copulations. In such situations, the best male strategy would probably be to deposit a plug at each mating against the possibility of interruption, but if no interruption occurs, to continue copulating.

Data on the actual effectiveness of copulatory plugs in foiling subsequent, competing copulations are few. Martan and Shepherd (1976) used albino female *Cavia porcellus* mated with albino and colored males in reciprocal trials to determine paternity of resultant offspring. When copulatory plugs were left *in situ*, litters were sired exclusively by the first male. When copulatory plugs were removed after the first mating, litters were of mixed paternity. These results are obviously consistent with a chastity-enforcing function of plugs in this species. Adler and Zoloth (1970) performed similar experiments using albino female *Rattus norvegicus* and albino and pigmented males, but did not provide data on presence/absence of copulatory plugs. Where albino males were the first to mate, percentages of offspring sired by pigmented males declined with increasing time between competing copulations: from 66% (0.5 min.) to 23% (45-60 min.). Whether this inverse correlation was due to increasing efficacy of plugs with time, if plugs were in fact retained by the females in question, or to the longer opportunities provided albino sperm to fertilize ova without competition, is unknown. Percentages of offspring sired by the second male remained consistently high (74-97%), regardless of time, when pigmented males were first to mate. Adler and Zoloth (1970) speculated that spermatozoa of the highly inbred pigmented males may have been competitively inferior. As these authors provided no information on plug presence/absence, their



data are not easily interpretable in terms of chastity enforcement. The experiments certainly deserve to be repeated with paternities of litters compared between females in which plugs remain *in situ* and females from which plugs are removed. Copulatory plugs need only decrease the percentage of offspring sired by subsequent males, not necessarily prevent them from being sired at all, in order to be selected for. Additionally, very short intervals between competing copulations may not accurately represent the selective context within which plugs evolved: adaptations cannot always be expected to function, let alone function efficiently, in evolutionarily novel situations.

### COPULATORY PLUGS, ACCESSORY GLANDS AND COPULATORY BEHAVIOR

Hartung and Dewsbury (1978) found that males of three New World murid species, *Onychomys leucogaster*, *O. torridus* and *Tylosomys nudicaudus* do not secrete copulatory plugs, and, in contrast to seven plug-secreting species studied, have copulatory behaviors that include vaginal-penile locks of brief duration. Dewsbury (1972, 1975) had observed previously that murids with locking copulatory behaviors possess reduced or modified accessory gland complements (Table 3). As can be seen, accessory gland arrays of locking species are more often reduced or modified than are those of non-locking species. Whether all locking species also fail to secrete plugs is unknown; all non-locking species thus far studied secrete plugs. A full accessory gland complement appears to be the primitive condition for murid rodents (Voss and Linzey, in preparation); retention of such a full complement by all murids known to secrete plugs, and the loss or modification of glands in three plugless species lends credence to the hypothesis that plug secretion is an important function of these organs.

If the correlation between a locking copulatory behavior and absence of plug secretion results from a causal relationship between the two, then copulatory locks must serve much the same function(s) as the plugs they presumably replaced. As noted by Hartung (1976), it is difficult to see how brief locks could function in sperm storage, prevention of sperm leakage, or chastity enforcement. Hartung concluded they could function in sperm transport, since the thick penises of locking species might dilate the cervixes of females, which are tightly constricted in the three species he studied, to permit sperm entry into the uterus. If copulatory locks have functionally replaced plugs in these species, and if locking and non-locking species

TABLE 3.

## MALE ACCESSORY GLAND MORPHOLOGIES FOR SPECIES OF MURID RODENTS WITH KNOWN COPULATORY BEHAVIORS.

Abbreviations: A, ampullary gland; AP, anterior prostate; BU, bulbo-urethral gland; DP, dorsal prostate; V, vesicular gland; VP, ventral prostate (two lobes in some species). Morphology: X = present; X<sub>1</sub> = greatly enlarged; X<sub>v</sub> = vestigial; (X) = highly modified from presumed ancestral condition; 0 = absent; XX = two lobes present. Sources: (1) Dewsbury, 1975; (2) Arata, 1964; (3) Linzey and Layne, 1969; (4) Taylor, 1963; (5) Hartung, 1976; (6) Lawlor, 1969; (7) Asdell, 1964; (8) Helm, 1975; (9) Voss, unpublished data. Accessory gland nomenclature follows Arata (1964).

Species	Lock?	Plug?	BU	DP	VP	AP	V	A	Sources
<i>Tylomys nudicaudus</i>	yes	no	X <sub>1</sub>	X	X <sub>v</sub>	X	(X)	X	1,5,9
<i>Ototylomys phyllotis</i>	yes	yes?	X	0	X	0	(X)	X	1,6,8
<i>Onychomys torridus</i>	yes	no	X	X	X	0	0	0	1,4,5
<i>O. leucogaster</i>	yes	no	X	X	X	0	0	0	1,2,5
<i>Baiomys taylori</i>	yes	?	X	X	X	X	X	X <sub>v</sub>	1,2
<i>Ochrotomys nuttalli</i>	yes	?	X	X	XX	X <sub>v</sub>	X <sub>v</sub>	0	1,2
<i>Neotoma floridana</i>	yes	?	X	0	X	(X)	0	(X)	1,2
<i>Mus musculus</i>	no	yes	X	X	XX	X	X	X	1,2,7
<i>Rattus norvegicus</i>	no	yes	X	X	XX	X	X	X	1,2,7
<i>M. tristrami</i>	no	?	X	X	X <sub>v</sub> X <sub>v</sub>	X	X	X	1,5,9
<i>Meriones unguiculatus</i>	no	?	X	X	X <sub>v</sub> X <sub>v</sub>	X	X	X	1,9
<i>Mesocricetus auratus</i>	no	yes	X	X	X	X	X	X	1,2,5
<i>Peromyscus californicus</i>	no	?	X	X	X	X	X	X	1,3
<i>P. crinitus</i>	no	?	X	X	XX	X	X	X	1,3
<i>P. eremicus</i>	no	yes	X	X	XX	X	X	X	1,3,5
<i>P. floridanus</i>	no	?	X	X <sub>v</sub>	X <sub>v</sub> X <sub>v</sub>	X <sub>v</sub>	X <sub>v</sub>	X <sub>1</sub>	1,3
<i>P. gossypinus</i>	no	yes	X	X	XX	X	X	X	1,3,5
<i>P. leucopus</i>	no	yes	X	X	XX	X	X	X	1,3,5
<i>P. maniculatus</i>	no	yes	X	X	XX	X	X	X	3,5
<i>P. polionotus</i>	no	?	X	X	XX	X	X	X	1,3
<i>Microtus pennsylvanicus</i>	no	yes	X	X	XX	X	X	X	1,2,7
<i>M. pinetorum</i>	no	yes	X	X	XX	X	X	X	1,2,7
<i>Oryzomys palustris</i>	no	?	X	X	XX	X	X	X	1,2
<i>Sigmodon hispidus</i>	no	yes	X	X	XX	X	X	X	1,2,7

are subject to similar selective pressures, then it follows that facilitation of sperm transport may have been the ancestral function of rodent, or at least murid, plugs. However, the two species of *Onychomys* are the murids for which best evidence of long-term pair bonding and possible monogamy exist (Dewsbury and Jansen, 1972; Egoscue, 1960; Horner and Taylor, 1968; Ruffer, 1965a, 1965b, 1968). For these, an ancestral, chastity-enforcing function of the plug may have become obsolete, permitting a secondary (sperm-transport?) role to be replaced by a behavior that was less expensive metabolically than maintenance of an elaborate glandular array. No data are avail-

able on the social biology of the third known plugless species, *Tylomys nudicaudus*.

Because so little is actually known of the interactions of most accessory gland secretions, it is difficult to interpret the different accessory gland morphologies (Table 3) that apparently correlate with pluglessness. If plugs are formed from secretions of anterior prostates and vesiculars, then why are the remaining glands of the male tract often reduced or modified in the absence of plug deposition? Other glands do participate to a lesser extent in rodent plug formation, however; the bulbo-urethrals, for instance, have been clearly implicated in this regard (Hart and Greenstein, 1968). Gotterer *et al.*, (1955) found that the coagulation reaction *in vitro* is very sensitive to ion concentrations, suggesting that osmotically active substances and electrolytes secreted by glands of the rodent prostate series other than the anterior lobe may also be functionally related to plug formation.

#### FUNCTION *VERSUS* EFFECT AND A PROPOSED TEST

To identify the proper function of a presumed adaptation it is not enough to demonstrate an effect, one must also “. . . show that it [the effect] is produced by design and not by happenstance” (Williams, 1966:261). I would argue that a male secretion which congeals almost instantly upon ejaculation (Mann, 1964) to form a hard, resistant mass completely occluding the vagina to which it adheres so tightly upon further, normal hardening that loss of epithelial layers is necessary to effect removal does not display those design characteristics one would expect of an evolved mechanism for inducing pseudopregnancy or facilitating sperm transport. I do not deny that copulatory plugs may have the effect of inducing luteal activity or transporting sperm in some rodents, but I suggest these are effects only, and not the primary function of plugs. Once copulatory plugs had evolved as a normal concomitant of successful ejaculations, one might expect that females would evolve to employ the stimulus of plug formation as the cue to initiate such responses as orgasm and/or luteal activation. Thus, while these effects, once established, could provide significant stabilizing selection on the genes responsible for plug formation, they seem inappropriate choices as the selective pressures that elicited plug evolution, quite aside from the data which I have already cited in their disfavor. Sperm storage and prevention of sperm leakage seem at least credible functions of the copulatory plug given its design characteristics, but the weight of evidence does not appear to be in their favor either. By contrast, the

general characteristics of rodent plugs seem to be consistent with a hypothesized role in chastity enforcement. Copulatory plugs are completely effective in preventing the spermatozoa of subsequent matings from effecting fertilization in *Cavia porcellus*, but data from *Rattus norvegicus* are equivocal in this regard. How, then, might the chastity-enforcement hypothesis be falsified?

I believe the critical test situation is to be found among those murid species known *not* to secrete plugs, and for which it can be stated with some certainty that the lack of plug secretion is derived. The chastity-enforcement hypothesis clearly predicts that such species should prove largely monogamous, i.e., should display substantially greater mating exclusivity than most murids that secrete plugs. Three plugless species, as discussed above, are known; field and laboratory data, admittedly indirect, strongly suggest that two of these form unusually cohesive pair bonds. Convincing tests of the chastity-enforcement hypothesis, however, must rely on direct observations of breeding animals under natural or seminatural conditions (e.g., Calhoun, 1963; Kenagy, 1976; Rood, 1970), perhaps in conjunction with electrophoretic mother-offspring comparisons that can distinguish between single and multiple matings; interpretation of the behavior of caged animals is usually fraught with too many assumptions to be admitted as conclusive in this regard. If future studies fail to corroborate the chastity-enforcement hypothesis, then another, equally parsimonious hypothesis of plug function should be advanced and tested in turn. In particular, it should be shown that (1) the selective pressure hypothesized to have elicited plug evolution in fact exists, and (2) copulatory plugs display efficient and appropriate design to produce the relevant effect. Much existing work on rodent copulatory plugs fails in one or both of these regards.

Table 4 presents a possible sequence of evolutionary stages that could have led to the loss of copulatory plugs and accessory gland reduction in some species of murid rodents. Stage One may be taken to represent the probable ancestral murid condition. The primitive status of a 'complete' accessory gland complement is argued elsewhere (Voss and Linzey, in preparation). That a non-locking copulatory behavior and the secretion of plugs are also likely to prove ancestral seems reasonable since these appear by far the most widespread conditions in both murid and non-murid rodents (see above, and Dewsbury, 1972). That promiscuity is the primitive social milieu for murids is an implicit assumption of the chastity-enforcement hypothesis which does not appear unreasonable for reasons I have adduced

**TABLE 4.**  
A HYPOTHETICAL SEQUENCE FOR THE EVOLUTION OF  
REDUCED GLANDULAR COMPLEMENTS AND LOSS OF  
COPULATORY PLUGS IN SOME MURID RODENTS.

STAGE	accessory glands	copulatory behavior	breeding system	copulatory plug	comments
one	complete	no lock	promiscuous	present	Copulatory plug functions primarily in chastity enforcement, secondarily effects sperm transport past cervix.
two	complete	no lock	incipient monogamy	present	Obsolescence of chastity enforcing function of plug.
three	complete	incipient lock	monogamy	present	Chastity enforcement obsolete; lock begins to assume secondary, sperm-transport role of plug.
four	reduced	lock	monogamy	absent	Sperm transport role completely assumed by lock; accessory glands now provide medium for sperm suspension only.

above. Remaining stages follow within the context of the chastity-enforcement hypothesis. It would be naive to imagine that Table 4 illustrates a deterministic or necessary sequence of events; I claim only that it is logical and consistent with existing data. I have assumed that some copulatory cue was necessary to induce orgasm and/or effect cervical dilation in the ancestral female murid, and that the vaginal-penile lock has this function in some extant, plug-less species, as suggested by Hartung (1976), but future studies may render this assumption untenable; it is, in any case, irrelevant to the chastity-enforcement hypothesis, and is included merely as an inde-

pendent hypothesis that might account for the apparent correlation between locking and pluglessness in murid rodents.

### SUMMARY

Many rodents possess an extraordinarily complex array of male accessory reproductive glands; considerable variability in this organ system also exists among some species of the family Muridae. This complexity is attributed to the secretion of a hard copulatory plug which occludes the vagina of the female. Five functions of copulatory plugs have been advanced: plugs may (1) store sperm, (2) prevent sperm leakage, (3) induce pseudopregnancy, (4) effect sperm transport or (5) prevent fertilization of the female by subsequent males. Of these, the last, chastity enforcement, appears most consistent with existing data; the remaining functions seem either unsupported by convincing evidence and/or best regarded as incidental effects. A realistic test of the chastity-enforcement hypothesis is proposed: rodent species which do not secrete plugs should be monogamous in nature. What is known of the biology of two such species is consistent with this prediction.

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### LITERATURE CITED

- ADLER, N. T. 1969. Effects of the male's copulatory behavior on successful pregnancy of the female rat. *J. Comp. Physiol. Psychol.* 69:613-622.  
ADLER, N. T., and S. R. ZOLOTH. 1970. Copulatory behavior can inhibit pregnancy in female rats. *Science* 168:1480-1482.  
ARATA, A. A. 1964. The anatomy and taxonomic significance of the male accessory reproductive glands of muroid rodents. *Bull. Fla. State Mus. Biol. Sci.* 9 (1):42pp.

- ASDELL, S. A. 1946. Patterns of mammalian reproduction, 1st edition. Comstock, Ithaca. 437pp.
- ASDELL, S. A. 1964. Patterns of mammalian reproduction, 2nd edition. Comstock, Ithaca. 670pp.
- ASDELL, S. A. 1966. Evolutionary trends in physiology of reproduction, pp. 1-13. In: I. W. Rowlands, ed. Comparative biology of reproduction in mammals. Symp. Zool. Soc. Lond. 15. Academic Press, London. 559pp.
- BALL, J. 1934. Demonstration of a quantitative relationship between stimulus and response in pseudopregnancy in the rat. *Am. J. Physiol.* 107: 698-703.
- BARNES, G. W. 1972. The antigenic nature of male accessory glands of reproduction. *Biol. Reprod.* 6:384-421.
- BENTLEY, A. J., and A. L. SODERWALL. 1939. Observations on germ cell viability and fertility in the female hamster reproductive tract. *J. Gerontol.* 8:373-374.
- BENTON, A. H. 1955. Observations on the life history of the northern pine mouse. *J. Mammal.* 36:52-62.
- BIRDSALL, D. A., and D. NASH. 1973. Occurrence of successful multiple insemination of females in natural populations of deer mice (*Peromyscus maniculatus*). *Evolution* 27:106-110.
- BISHOP, D. W. 1961. Biology of spermatozoa, pp. 707-796. In: W. C. Young and G. W. Corner, eds. Sex and internal secretions, 3rd edition. Williams and Wilkins, Baltimore. 1609pp.
- BLANDAU, R. J. 1945. On the factors involved in sperm transport through the cervix uteri of the albino rat. *Am. J. Anat.* 77:253-272.
- BLANDAU, R. J. 1969. Gamete transport—comparative aspects, pp. 129-162. In: E. S. E. Hafez and R. J. Blandau, eds. The mammalian oviduct. Univ. Chicago Press, Chicago. 546pp.
- BLANDAU, R. J. 1973. Sperm transport through the mammalian cervix: comparative aspects, pp. 285-304. In: R. J. Blandau and K. Moghissi, eds. The biology of the cervix. Univ. Chicago Press, Chicago. 450pp.
- BLANDAU, R. J., and D. L. ODOR. 1949. The total number of spermatozoa reaching various segments of the reproductive tract in the female albino rat at intervals after insemination. *Anat. Rec.* 103:93-109.
- BLANDAU, R. J., and W. C. YOUNG. 1939. The effects of delayed fertilization on the development of the guinea pig ovum. *Am. J. Anat.* 64:303-329.
- BRAMBELL, F. W. R., and K. HALL. 1939. Reproduction of the field vole, *Microtus agrestis hirtus* Bellamy. *Proc. Zool. Soc. Lond.* 109A: 133-138.
- BRAMBELL, F. W. R., and I. W. ROWLANDS. 1936. Reproduction of the bank vole (*Evotomys glareolus* Schreber) I. the oestrous cycle of the female. *Philos. Trans. R. Soc. Lond. B Biol. Ser.* 226:71-98.
- BUTTERWORTH, B. B. 1961. The breeding of *Dipodomys deserti* in the laboratory. *J. Mammal.* 42:413-419.
- CALHOUN, J. B. 1963. The ecology and sociology of the Norway rat. U. S. Department of Health, Education and Welfare, Bethesda. 288 pp.
- CLARKE, J. R., F. V. CLULOW and F. GRIEG. 1970. Ovulation in the bank vole, *Clethrionomys glareolus*. *J. Reprod. Fert.* 23:531. (Abstr.)
- CONAWAY, C. H. 1971. Ecological adaptation and mammalian reproduction. *Biol. Reprod.* 4:239-247.
- CROSS, P. C. 1972. Observations on the induction of ovulation in *Microtus montanus*. *J. Mamm.* 53:210-212.

- DEANESLEY, R., and A. S. PARKES. 1933. The oestrous cycle of the grey squirrel (*Sciurus carolinensis*). Philos. Trans. R. Soc. Lond. B Biol. Ser. 222:47-78.
- DEVINE, M. 1975. Copulatory plugs in snakes: enforced chastity. Science 187:844-845.
- DEVINE, M. 1977. Copulatory plugs, restricted mating opportunities and reproductive competition among male garter snakes. Nature 267:345-346.
- DEWSBURY, D. A. 1972. Patterns of copulatory behavior in male mammals. Q. Rev. Biol. 47:1-33.
- DEWSBURY, D. A. 1975. Diversity and adaptation in rodent copulatory behavior. Science 190:947-954.
- DEWSBURY, D. A. 1976. Copulatory behavior of white-footed mice (*Peromyscus leucopus*) in a multimale situation. Bull. Psychon. Soc. 7:340-342.
- DEWSBURY, D. A., and P. E. JANSEN. 1972. Copulatory behavior of southern grasshopper mice (*Onychomys torridus*). J. Mammal. 53:267-278.
- EADIE, W. R. 1948. Corpora amyloacea in the prostatic secretion and experiments on the formation of a copulatory plug in some insectivores. Anat. Rec. 102:259-267.
- EGOSCUE, H. J. 1960. Laboratory and field studies of the northern grasshopper mouse. J. Mammal. 41:99-110.
- EISENBERG, J. F. 1966. The social organization of mammals. Handbuch der Zoologie VII (10/7) Lieferung 39:92pp. De Gruyter, Berlin.
- ENGLE, E. T. 1925. Exfoliation in toto of the vaginal mucosa in the guinea pig. Anat. Rec. 29:383. (Abstr.)
- ENGLE, E. T. 1926a. A morphological and experimental study of the proximal lobes of the prostate of the guinea pig, *Cavia cobaya*. Anat. Rec. 34:75-90.
- ENGLE, E. T. 1926b. The copulation plug and the accessory genital glands of mammals. J. Mammal. 7:119-126.
- GOTTERER, G., D. GINSBURG, T. SCHULMAN, J. BANKS, and H. G. WILLIAMS-ASHMAN. 1955. Enzymatic coagulation of semen. Nature 176:1209-1211.
- GREENSTEIN, J. S., and R. G. HART. 1964. The effect of removal of the accessory glands separately or in paired combinations on the reproductive performance of the male rat. Fifth Int. Congr. Anim. Reprod. Artif. Insem. Trento 3:414-420.
- HAMILTON, W. J. 1941. Reproduction of the field mouse *Microtus pennsylvanicus* (Ord). Cornell Univ. Agric. Exp. Station Mem. 237: 23pp.
- HART, R. G., and J. S. GREENSTEIN. 1968. A newly discovered role for Cowper's gland secretion in rodent semen coagulation. J. Reprod. Fert. 17:87-94.
- HARTMAN, C. G. 1924. Observations on the motility of the opossum genital tract and the vaginal plug. Anat. Rec. 27:293-303.
- HARTMAN, C. G., and J. BALL. 1930. On the almost instantaneous transport of spermatozoa through the cervix and the uterus in the rat. Soc. Exp. Biol. Med. 28:312-314.
- HARTUNG, T. G. 1976. A comparative analysis of copulatory plugs in rodents and their relationship to copulatory behavior. Unpublished Masters thesis, Univ. Florida. 47pp.
- HARTUNG, T. G., and D. A. DEWSBURY. 1978. A comparative analysis of copulatory plugs in muroid rodents and their relationship to copulatory behavior. J. Mammal. 59:717-723.



- HELM III, J. D. 1975. Reproductive biology of *Ototylomys* (Cricetidae). *J. Mammal.* 56:575-590.
- HOOPER, E. T., and G. G. MUSSER. 1964. The glans penis in neotropical cricetines (Muridae) with comments on classification of muroid rodents. *Misc. Publ. Mus. Zool. Univ. Michigan* 123:57 pp.
- HORNER, B. E., and J. M. TAYLOR. 1968. Growth and reproductive behavior in the southern grasshopper mouse. *J. Mammal.* 49:644-660.
- KENAGY, G. J. 1976. Field observations of male fighting, drumming, and copulation in the Great Basin Kangaroo rat, *Dipodomys microps*. *J. Mammal.* 57:781-785.
- KENT, G. C. 1968. Physiology of reproduction, pp. 119-138. *In*: R. A. Hoffman *et al*, eds. The golden hamster, its biology and use in medical research. Iowa State Univ. Press, Ames, Iowa. 545pp.
- KITCHENER, D. J. 1975. Reproduction in female Gould's bat, *Chalinobus gouldii* (Gray) (Vespertilionidae), in western Australia. *Aust. J. Zool.* 23:29-42.
- KLEIMAN, D. G. 1977. Monogamy in mammals. *Q. Rev. Biol.* 52:39-69.
- KOREN, E., J. LUKAC, and S. MILKOVIC. 1974. The effect of collagen-like peptidase from rat testis and clostridial collagenase A on the rat seminal vesicle secretion and its coagulation. *J. Reprod. Fert.* 36:161-167.
- KOREN, E., E. SCHON, and J. LUKAC. 1975. The coagulation of insoluble and basic proteins from rat seminal vesicle secretion with vesiculase: influence of collagenase-like peptidase from rat testis. *J. Reprod. Fert.* 42:491-495.
- LAND, R. B., and T. E. MCGILL. 1967. The effects of the mating pattern of the mouse on the formation of corpora lutea. *J. Reprod. Fert.* 13:121-125.
- LAWLOR, T. E. 1969. A systematic study of the rodent genus *Ototylomys*. *J. Mammal.* 50:28-42.
- LEUCKART, R. 1847. Zur Morphologie und Anatomie der Geschlechtsorgane. Göttingen. (not seen)
- LINZEY, A. V., and J. N. LAYNE. 1969. Comparative morphology of the male reproductive tract in the rodent genus *Peromyscus* (Muridae). *Am. Mus. Novit.* 2355:47pp.
- LISK, R. D. 1969. Cyclic fluctuations in sexual responsiveness in the male rat. *J. Exp. Zool.* 171:313-320.
- LONG, J. A. 1919. The oestrous cycle in rats. *Anat. Rec.* 15:352. (Abstr.)
- LONG, J. A., and H. M. EVANS. 1922. The oestrous cycle in the rat and its associated phenomena. *Mem. Univ. Calif.* 6:148pp.
- MANN, T. 1964. The biochemistry of semen and of the male reproductive tract. Methuen and Co., London. 493pp.
- MANN, T. 1974. Secretory function of the prostate, seminal vesicle and other male accessory organs of reproduction. *J. Reprod. Fert.* 37:179-188.
- MANN, T., and C. LUTWAK-MANN. 1951. Secretory function of male accessory sex organs of reproduction in mammals. *Physiol. Rev.* 31:27-55.
- MARTAN, J., and B. A. SHEPHERD. 1976. The role of the copulatory plug in reproduction of the guinea pig. *J. Exp. Zool.* 196:79-83.
- MCGILL, T., D. M. CORWIN, and D. T. HARRISON. 1968. Copulation plug does not induce luteal activity in the mouse *Mus musculus*. *J. Reprod. Fert.* 15:149-151.
- MERRITT, R. B., and B. J. WU. 1975. On the quantification of promiscuity (or "*Promyscus maniculatus*?). *Evolution* 29:575-578.

- MERTON, H. 1939. Studies on reproduction in the albino mouse. III. The duration of life of the spermatozoa in the female tract. Proc. R. Soc. Edinb. 59:207-218.
- MEYER, B. J., and R. K. MEYER. 1944. Growth and reproduction of the cotton rat, *Sigmodon hispidus hispidus*, under laboratory conditions. J. Mammal. 25:107-129.
- MILIGAN, S. R. 1975. The copulatory behavior of *Microtus agrestis*. J. Mammal. 56:220-224.
- MOSIG, D. W., and D. A. DEWSBURY. 1970. Plug fate in the copulatory behavior of rats. Psychon. Sci. 20:315-316.
- PARKER, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. Camb. Philos. Soc. 45:525-567.
- PARKES, A. S. 1926. Observations on the oestrous cycle of the albino mouse. Proc. Zool. Soc. Lond. 100B:151-170.
- PEARSON, O. P. 1949. Reproduction of a South American rodent, the mountain viscacha. Am. J. Anat. 84:143-174.
- PRICE, D. 1963. Comparative aspects of development and structure in the prostate, pp. 1-27. In: E. P. Vollmer, ed. The biology of the prostate and related tissues. Natl. Cancer Inst. Monograph 12:446pp.
- PRICE, D., and H. G. WILLIAMS-ASHMAN. 1961. The accessory reproductive glands of mammals, pp. 366-448. In: W. C. Young, ed. Sex and internal secretions, 3rd edition, vol. 1. Williams and Wilkins, Baltimore. 704pp.
- RACEY, P. A. 1975. The prolonged survival of spermatozoa in bats, pp. 385-416. In: J. G. Duckett and P. A. Racey, eds. The biology of the male gamete. Biol. J. Linn. Soc. London 7: Supplement No. 1.
- RICHMOND, M., and C. H. CONAWAY. 1969a. Induced ovulation and oestrous in *Microtus ochrogaster*. J. Reprod. Fert. Suppl. 6:357-376.
- RICHMOND, M., and C. H. CONAWAY. 1969b. Management, breeding, and reproductive performance of the vole, *Microtus ochrogaster* in a laboratory colony. Lab. Anim. Care 19:80-87.
- ROOD, J. P. 1970. Ecology and social behavior of the desert cavy (*Microcavia australis*). Am. Midl. Nat. 83:415-454.
- RUFFER, D. G. 1965a. Burrows and burrowing behavior of *Onychomys leucogaster*. J. Mammal. 46:241-247.
- RUFFER, D. G. 1965b. Sexual behavior of the northern grasshopper mouse (*Onychomys leucogaster*). Anim. Behav. 13:447-452.
- RUFFER, D. G. 1968. Agonistic behavior of the northern grasshopper mouse (*Onychomys leucogaster breviauritus*). J. Mammal. 49:481-487.
- RUGH, R. 1968. The mouse, its reproduction and development. Burgess, Minneapolis. 430pp.
- SNELL, C. C. 1956. Reproduction, pp. 55-58. In: C. C. Snell, ed. Biology of the laboratory mouse. Dover, New York. 497pp.
- SODERWALL, A. L., and R. J. BLANDAU. 1941. The duration of the fertilizing capacity of spermatozoa in genital tract of the rat. J. Exp. Zool. 88:55-64.
- SODERWALL, A. L., and W. C. YOUNG. 1940. The effect of aging in the female genital tract on the fertilizing capacity of guinea pig spermatozoa. Anat. Rec. 78:19-29.
- SPEYER, J. 1959. Semen coagulase, a new enzyme. Fed. Proc. 18:150. (Abstr.)
- STOCKARD, C. R., and G. N. PAPANICOLAOU. 1919. The vaginal closure membrane, copulation, and the vaginal plug in the guinea pig with further considerations of the oestrous rhythm. Biol. Bull. (Woods Hole) 37:222-245.

- TAYLOR, J. M. 1963. Reproductive mechanisms of the male grasshopper mouse. *J. Exp. Zool.*, 154:109-124.
- TINKLEPAUGH, O. O. 1930. Occurrence of a vaginal plug in a chimpanzee. *Anat. Rec.* 46:329-332.
- VON WAGENEN, G. 1936. The coagulating function of the prostate gland in the monkey. *Anat. Rec.* 66:411-421.
- WALKER, G. 1910. A special function discovered in a glandular structure hitherto supposed to form a part of the prostate gland in rats and guinea pigs. *Johns Hopkins Bull.* 21:182-185.
- WALTON, A. 1960. Copulation and natural insemination, pp. 130-160. *In*: A. S. Parkes, ed. *Marshall's physiology of reproduction*, 3rd edition, vol. 1, part 2. Longmans, London. 877pp.
- WEINERTH, J. L., C. R. BATTAGLIA, and H. MAGALHAES. 1961. The non-essential nature of seminal vesicles in golden hamsters. *Amer. Zool.* 1:397. (Abstr.)
- WIER, B. J. 1971. The reproductive physiology of the plains viscacha, *Lagostomus maximus*. *J. Reprod. Fert.* 25:355-363.
- WIER, B. J. 1974. Reproductive characteristics of hystricomorph rodents, pp. 265-299. *In*: I. W. Rowlands and B. J. Wier, eds. *The biology of hystricomorph rodents*. Symp. Zool. Soc. Lond. 34. Academic Press, London. 482pp.
- WIER, B. J., and I. W. ROWLANDS. 1974. Functional anatomy of the hystricomorph ovary, pp. 303-332. *In*: I. W. Rowlands and B. J. Wier, eds. *The biology of hystricomorph rodents*. Symp. Zool. Soc. Lond. 34. Academic Press, London. 482pp.
- WILLIAMS, G. C. 1966. *Adaptation and natural selection*. Princeton Univ. Press, Princeton. 307pp.

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