Responses of male *Rhabdomys pumilio* to urine of females in different reproductive states

Lara N. Bennett

Neville Pillay

Introduction

Olfactory signals are commonly used to indicate the reproductive status of female mammals, and may have highly specific effects on the sexual behaviour of males, such as attraction or eliciting sexual behaviour (FERKIN and JOHNSTON, 1995; JOHNSTON, 1983). For example, odour of a female during oestrus is one of the primary stimuli indicating her receptivity to males (EISENBERG and KLEIMAN, 1972). In many rodents, such as house mice *Mus musculus* (FERON and BAUDOIN, 1992), brown lemmings *Lemmus sibiricus* (HUCK and BANKS, 1984), woodrats *Neotoma lepida lepida* (FLEMING *et al.*, 1981), Djungarian hamsters *Phodopus campbelli* (LAI *et al.*, 1996) and rats *Rattus norvegicus* (NYBY, 1982), males spend greater time sniffing odours of oestrous than non-oestrous females.

Preference for oestrus over non-oestrus females is not universal, however. Male golden hamsters *Mesocricetus auratus* show no preference for odour of oestrous to dioestrous female conspecifics, but prefer odours of oestrous females to those of pregnant females (JOHNSTON, 1980). In some mammals, for example Djungarian

hamsters *P. campbelli* (LAI *et al.*, 1996), males are particularly attracted to the scent of postpartum oestrus females. This attraction is probably consistent with the situation in nature, since many mammals go through cycles of pregnancy rather than through repeated oestrous cycles (BRONSON, 1985).

In a recent study, BENNETT (1999) showed that male striped mice *Rhabdomys pumilio* are more attracted to the smell of urine than other odour sources (i.e. body and facial odour, saliva and vaginal secretions) of oestrous females. Nothing is known about whether male striped mice show differential preference of urinary odour over a variety of female reproductive states, which was the objective of the present study. In order to facilitate mating, receptive females are likely to produce substances in the urine which are attractive to males. Hence, it is predicted that male striped mice would prefer urine of receptive females (i.e. oestrus, postpartum oestrus) to non-receptive females (i.e. dioestrus and pregnant).

Rhabdomys pumilio is widely distributed in southern Africa (SKINNER and SMITHERS, 1990), occurring in high population densities in suitable habitats (WILLAN and MEESTER, 1989). It is a seasonal breeder, producing litters averaging around five pups (PERRIN, 1980; WILLAN and MEESTER, 1989). During the breeding season, adult females are intrasexually territorial with a limited home range size, and adult males occupy larger home ranges that overlap those of several females (PILLAY, 1999; SKINNER and SMITHERS, 1990). Mature females have a spontaneous oestrous cycle of four days, in which they are receptive for one day (oestrus; BENNETT, 1999). Females also have postpartum oestrus, mating within a day or two of parturition (PILLAY, 2000).

Materials and methods

Rhabdomys pumilio individuals were derived from wild caught parents from Alice, Eastern Cape Province (32° 48' S; 26° 52' E), South Africa. Thirty-two adult male and 38 adult female *R. pumilio* were used in the study. All animals were sexually experienced. They were housed

individually in Labotec® cages, under partially controlled environmental conditions at a temperature of approximately 25°C, rH = 30-50%, and a light regime of 14L; 10D (lights on between 05.00 and 19.00), thereby approximating summer in the southern hemisphere and hence optimal breeding conditions. Animals were fed ad libitum Epol® mouse cubes (containing 18% protein), and were provided twice weekly with maize and sunflower seeds. Water was available at all times.

Stimulus samples (urine) were collected from donor females in five different reproductive states: i) oestrus (cycling, receptive females); ii) dioestrus (cycling, non-receptive females); iii) mid-pregnancy (females in their second week of pregnancy, approximately mid-term of the 23-day gestation period); iv) late-pregnancy (2-3 days before parturition); v) postpartum oestrus (12-48 hours after parturition). Vaginal smears were taken to establish postpartum oestrus as well as the stage of oestrus of cycling females. Mated females were assessed for pregnancy by means of vaginal smears and palpation.

Urine samples from females in the different reproductive states were presented to test males in seven different treatments in two-choice tests: i) dioestrus vs oestrus; ii) dioestrus vs mid-pregnant; iii) dioestrus vs late pregnant; iv) dioestrus vs postpartum oestrus; v) oestrus vs mid-pregnant; vi) oestrus vs late pregnant; and vii) oestrus vs postpartum oestrus. Twelve replicates of each treatment were performed. Before conducting experiments, 12 control tests were carried out, where males were offered a choice of two clean cotton buds. This was done to expose any potential bias in test equipment.

Of the 32 males used in experiments, four were initially tested per treatment and the control. Males were then re-used twice more in treatments or control tests. No male was exposed to the same treatment or the control more than once, and they were never exposed to the urine of a particular female more than once. The allocation of males to the experiments was done randomly. Males were allowed a rest period of three days between consecutive tests.

Tests were carried out by first collecting urine from two scent donor females by wiping a clean cotton bud over the female's clitoris as she produced urine. The cotton buds (with the urine samples) were then placed into clean petri dishes, covered with gauze, and placed into a two-choice chamber. The choice chamber comprised three linearly

arranged compartments. The stimulus samples were randomly placed into the two outer compartments, while the test male was initially placed in the centre compartment. During experiments, he had access to the urine samples by means of interconnecting passages; a complete description and illustration of the choice chamber is provided in PILLAY *et al.* (1995).

The behaviour of the male was then recorded using a Sony TR880E video camera. All tests were conducted during the light phase of the light-dark cycle, since *R. pumilo* is diurnal.

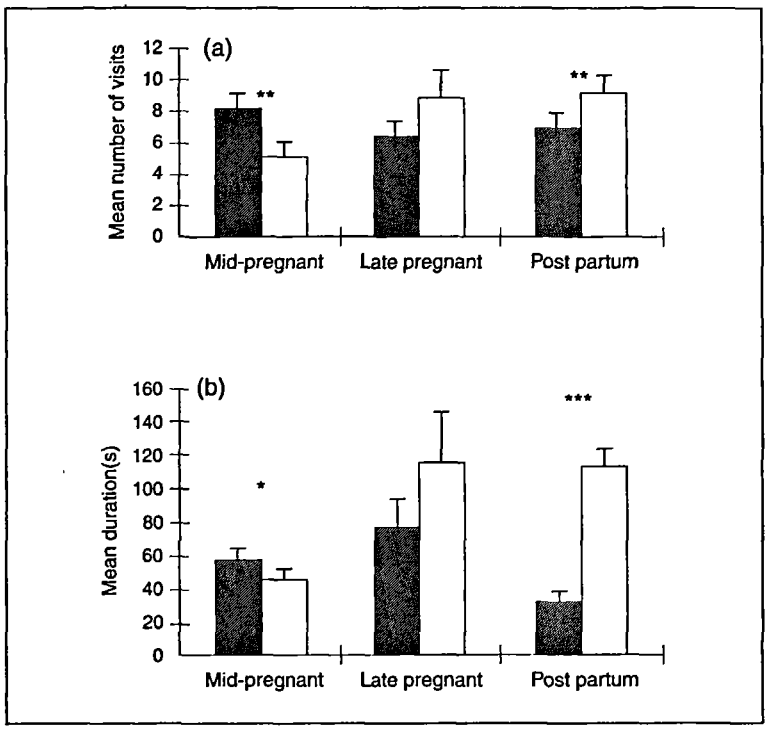
Immediately after the male made contact with either one of the urine samples, the total time he spent at each urine sample, and number of times he visited each urine sample were recorded for 20 min. The mean duration of time spent at each urine sample and the mean number of visits to each urine sample were calculated per combination of reproductive states. Data were analysed using a two-tailed paired-t test to establish whether males displayed a directional preference in each treatment and in the control (ZAR, 1996).

Results

Males spent an equal duration at both left and right control samples ($p > 0.05$; paired t-test), suggesting no bias in test equipment.

In experiments involving choice between dioestrous and oestrous female urine samples, the frequency of visits by males to dioestrous ($X \pm SE = 7.50 \pm 1.02$) and oestrous (7.30 ± 0.98) samples did not differ significantly ($p > 0.05$, paired t-test). Males spent equivalent amounts of time at oestrous (54.00 ± 6.96 s) and dioestrous (49.20 ± 8.24 s) female urine samples ($p > 0.05$; paired t-test).

The mean number of visits and the duration of visits of males to dioestrous female urine samples versus those from females in different stages of pregnancy or postpartum oestrus are shown in Figure 1. Males did not show a clear preference between urine of dioestrous females and that of females in late pregnancy, but preferred dioestrus

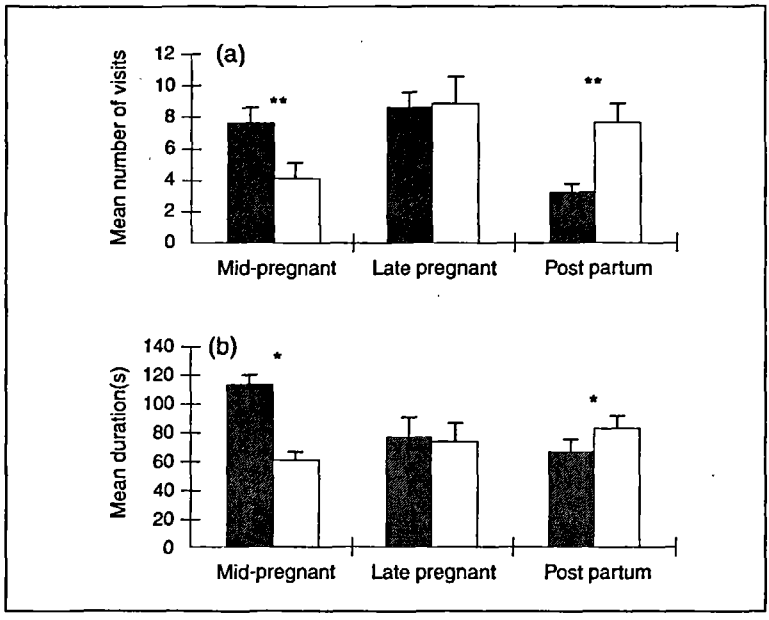


*p < 0.05; **p < 0.01; ***p < 0.001.

Figure 1
Mean number of visits (a) and mean duration (b) displayed by male *R. pumilio* in respect of urine samples from dioestrous females (black bars) versus those from females in other reproductive stages (open bars).
Error bars = 1SE of the mean.

to mid-pregnant female urine (fig. 1). Males clearly preferred urine of postpartum oestrous females to urine of dioestrous females (fig. 1).

Figure 2 gives the number of visits and the duration of visits that males made to urine from oestrous females to those in different stages of pregnancy and postpartum oestrus. Males significantly preferred the urine of oestrous females to that of mid-pregnant females. Postpartum oestrous female odour was preferred to oestrous female odour (fig. 2).



*p <0.05, **p < 0.01.

Figure 2
Mean number of visits (a) and mean duration (b) displayed by male *R. pumilio* in respect of urine samples from oestrous females (hatched bars) versus those females in other reproductive stages (open bars). Error bars = 1SE of the mean.

Discussion

The prediction that male *R. pumilio* would prefer receptive to non-receptive females was only partially supported in this study. On the one hand, males showed equal preference for dioestrous and oestrous female urine, and for cycling females and those in late pregnancy. On the other hand, urine from cycling females was preferred to that of females in mid-pregnancy. In addition, postpartum oestrous female urine was highly attractive to *R. pumilio* males, significantly more so than urine from cycling females.

The literature on rodent olfactory communication shows both similarities and differences to the results obtained in the present study.

Male golden hamsters *M. auratus* showed no preference for odours of dioestrous or oestrous females, but preferred the odours of oestrous females over those of pregnant or lactating females (golden hamsters do not have a postpartum oestrus; JOHNSTON, 1980). Male Djungarian hamsters *P. campbelli* were more attracted to urine from females in postpartum oestrus than that of dioestrous females, but showed no preference for urine from females the day before parturition (late-pregnancy) or from dioestrous females (LAI *et al.*, 1996). In meadow voles *Microtus pennsylvanicus,* male attraction to female scent increased greatly just after parturition when females were in post-partum oestrus (FERKIN & JOHNSTON, 1995).

The lack of preference between the urine of dioestrous females and oestrous females urine by male striped mice should be interpreted with caution, since such a situation could imply either an inability to discriminate between the scents or an equal interest in both urine samples. In recent experiments (PILLAY unpubl. data), however, male *R. pumilio* were sexually aroused by the presence of oestrous females and not dioestrous females, displaying more mounting and nasal-anal behaviours in the presence of oestrous females. Hence, males can discriminate between the two reproductive states. This does raise the possibility that a preference for oestrous females would occur when a greater number of signals is available. In our study, males could potentially evaluate the stimuli with the sense of smell (olfactory and vomeronasal) only, but other cues could elicit differential responses in the male; for example, ultrasonic calls or lordotic posture of females (TAYLOR & DEWSBURY, 1990).

LAI *et al.* (1996) suggest that some pregnant female rodents might produce a "stay away" signal that males find unattractive. Similarly, the reduced interest in mid-pregnant female urine corresponds with the time when females are furthest away from receptivity. It is intriguing therefore that the urine of females in late pregnancy (a few days away from receptivity) was as attractive to males as the urine of both dioestrous and oestrous females. These results may be explained by the social and population biology of *R. pumilio.*

Striped mice occur at high densities during the breeding season, and male-male competition for breeding females is apparently intense (WILLAN & MEESTER, 1989). Females are receptive within two days of both dioestrous and late pregnancy (2-3 days before parturition in

the present study). Thus, it would be advantageous for males to be equally attracted to females in dioestrous and late-pregnancy, as they are to oestrous females, and remain near these females shortly before receptivity (DEWSBURY *et al.*, 1986; JOHNSTON, 1980).

The preference for the urine of postpartum females over that of cycling females is consistent with the reproductive biology of the species. Free-living *R. pumilio* reproduce at a high rate (BROOKS, 1982; PERRIN, 1980; WILLAN and MEESTER, 1989) which means females would be pregnant or in postpartum oestrus throughout the breeding season. Females would therefore go through cycles of pregnancy rather than oestrous cycles, and males are more likely to encounter postpartum oestrous females than cycling females.

Further studies involving chemical analysis of *R. pumilio* urine should be done to evaluate the changes in components and/or concentrations of components that may exist between various female reproductive stages.

Acknowledgements

Thanks to Rod Baxter for providing animals from Alice, David Amm, Dineshree Padiachy, Ute Schwaibold and staff at the Central Animal Service for technical assistance. Financial support from the University of the Witwatersrand is highly appreciated. Animal ethics clearance number: AESC 98/101/2a.

References

BENNETT L.N., 1999 — Chemical communication in the water rat *Dasymys incomtus* and striped mouse *Rhabdomys pumilio*: discrimination of oestrous condition by male conspecifics. M.Sc. thesis, Univ. of the Witwatersrand, Johannesburg, 105 p.

BRONSON F.H., 1985 — Mammalian reproduction: an ecological perspective. *Biology of Reproduction*, 32: 1-26.

BROOKS P.M., 1982 — Aspects of the reproduction, growth and development of the four-striped field mouse, *Rhabdomys pumilio* (Sparrman, 1784). *Mammalia*, 46: 53-64.

DEWSBURY D.A., FERGUSON B., HODGES A.W. and TAYLOR S.A., 1986 — Tests of preferences of deer mice (*Peromyscus maniculatis bairdi*) for individuals and their odors as a function of sex and estrous condition. *Journal of Comparative Psychology* 100: 117-127.

EISENBERG J.F. and KLEIMAN D.G., 1972 — Olfactory communication in mammals. *Annual Review of Ecology and Systematics* 3: 1-32.

FERKIN M.H. and JOHNSTON R.E., 1995 — Meadow voles, *Microtus pennsylvanicus*, use multiple sources of scent for sex recognition. *Animal Behaviour* 49: 37-44.

FERON C. and BAUDOIN C., 1992 — Reactions of staggerer and non-mutant male mice to female urine and vaginal secretion odours. *Behavioural Processes* 27: 165-170.

FLEMING A.S., CHEE P. and VACCARINO F., 1981 — Sexual behaviour and its olfactory control in the desert wood rat (*Neotoma lepida lepida*). *Animal Behaviour* 29: 727-745.

HUCK U.W. and BANKS E.M., 1984 — Social olfaction in male brown lemmings (*Lemmus sibiricus = trimucronatus*) and collared lemmings (*Dicrostonyx groenlandicus*): 1. Discrimination of species, sex and oestrous condition. *Journal of Comparative Physiologyy* 98: 54-59.

JOHNSTON R.E., 1980 — Responses of male hamsters to odours of females in different reproductive states. *Journal of Comparative Physiology*, 94: 894-904.

JOHNSTON R.E., 1983 — Chemical signals and reproductive behaviour. In VANDENBERGH, J.G. (ed.): *Pheromones and reproduction in mammals*, New York, Academic Press: 3-37.

LAI S.-C., VASILIEVA N.Y. and JOHNSTON R.E., 1996 — Odours producing sexual information in Djungarian hamsters: evidence for an across-odour code. *Hormones & Behaviour* 30: 26-36.

NYBY J., 1982 — Volatile and non-volatile chemosignals of female rodents: differences in hormonal regulation. In MULLER-SCHWARZE D. and SILVERSTEIN R.M. (eds): *Chemical signals in vertebrates* 3, New York, Plenum Press: 179-193.

PERRIN M.R., 1980 — The breeding strategies of two coexisting rodents, *Rhabdomys*

pumilio and *Otomys irroratus*: with a brief review of some pertinent life history ideas. *Acta Ecologica* 1: 383-410.

PILLAY N., 1999 — The effects of sibling reproduction in the African striped mouse. *Acta Theriologica* 44: 291-298.

PILLAY N., 2000 — Fostering in the African striped mouse: implications for kin recognition and dominance. *Acta Theriologica* 45: 00-00

PILLAY N., WILLAN K. and MEESTER, J., 1995 — Evidence of pre-mating reproductive isolation in two allopatric populations of the vlei rat (*Otomys irroratus*). *Ethology* 100: 61-71.

SKINNER J.D. and SMITHERS R.H.N., 1990 — *Mammals of the southern African subregion*. Univ. of Pretoria Press, Pretoria, 769 p.

TAYLOR S.A. and DEWSBURY D.A., 1990 — Male preferences for females of different reproductive conditions: a critical review. In MACDONALD D.W., MULLER-SCHWARZE D., NATYNCZUK S.E. (eds): *Chemical signals in vertebrates* 5, Oxford, Oxford Science Publications: 184-198.

WILLAN K. and MEESTER J.A.J., 1989 — Life history styles of southern African *Mastomys natalensis, Otomys irroratus* and *Rhabdomys pumilio* (Mammalia, Rodentia). In BRUTON M.N., (ed.): *Alternative life-history styles of animals*, Dordrecht, Kluwer academic Publishers: 421-439.

ZAR J. H., 1996 — *Biostatistical analysis*. 3rd edition. London, Prentice Hall, Inc., 662 p.