Reproductive biology of the Cape serotine bat, *Eptesicus capensis* in the Transvaal, South Africa

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The reproductive biology of the Cape serotine bat, *Eptesicus capensis*, was investigated histologically. The study was based on 67 specimens collected over a six-year period. This species is seasonally monoestrous, normally giving birth to twins during November. Spermatogenesis peaks during autumn (March–May) when masses of spermatozoa are released into the epididymides, and spermatozoa are present in the epididymides from March to October. Copulations are initiated during this period from the end of March to the beginning of April, with the first ovulations occurring during August. During the pre-ovulation period leucocytes are commonly found within the uterine lumen and uterine glands of females.

Die voortplantingsbiologie van die Kaapse dakvlermuis, *Eptesicus capensis*, is histologies ondersoek. Die studie is gebaseer op 67 monsters wat oor 'n periode van ses jaar versamel is. Hierdie spesie is seisoenaal monestrus, en skenk gewoonlik geboorte aan tweelinge gedurende November. Spermatogenese bereik 'n piek gedurende die herfs (Maart–Mei) wanneer massas sperme in die epididimi gestort word, en sperme is teenwoordig in die epididimi vanaf Maart tot Oktober. Kopulasie neem 'n aanvang gedurende die periode einde Maart en begin April, terwyl die eerste ovulasies gedurende Augustus plaasvind. Gedurende die pre-ovulasie periode is leukosiete algemeen in die uteruslumen en -kliere van wyfies gevind.

The Cape serotine bat *Eptesicus capensis* (A. Smith, 1829) is a member of the family Vespertilionidae, which is the largest family of the insectivorous bats (Hill & Smith 1984). Members of this family occur throughout the world, except in polar and near polar regions (Hill & Smith 1984).

In the Transvaal the Cape serotine bat is common and widespread with a wide habitat tolerance (Rautenbach 1982). It is a small species with males averaging 5,9 g and femals 7,3 g (Rautenbach 1982). Although they are quite common in the Transvaal, knowledge about their reproductive biology is limited. Rautenbach (1982) collected gravid females during October and November and lactating females during September, November and December. From these data he suggested a parturition season during the first half of summer. The number of foetuses varies from singletons to triplets (Shortridge 1934; Smithers 1971; Rautenbach 1982; Skinner & Smithers 1990). Amongst the vespertilionids twins are common (Hill & Smith 1984), with triplets exceptional (Stuart & Stuart 1988; Skinner & Smithers 1990).

The purpose of the present study is to investigate the reproductive biology of the Cape serotine bat.

Material and Methods

A total of 67 specimens (27 males, 40 females) were collected from the farm Klipfontein, 30 km north-east of Vaalwater in the Transvaal, South Africa (24°08'S / 28°18'E).

Owing to small sample sizes it was necessary to combine data from six years (1983, 1984, 1987, 1988, 1989, 1990, Table 1) to get a picture of the annual reproductive cycle of this species. All bats were captured using two 30×6 m mist-nets (Rautenbach 1985) and were brought to the laboratory alive where they were sacrificed using ether. Testes, epididymides, non-pregnant uteri and uteri with embryos were preserved in Bouin's fluid. Uteri containing foetuses were stored in AFA (a mixture of 95% ethyl alcohol, 40% formalin, glacial acetic acid and distilled water in a ratio of 3:1:1:5 by volume).

Testes, epididymides and uteri containing embryos were prepared for histological examination. Following paraffin-wax embedding all samples were serially sectioned at 5 µm and the mounted series stained with Ehrlich's haematoxylin and counterstained with eosin.

Results

Spermatogenic activity was initiated towards the end of spring. Three males collected on 6 November 1987 showed spermatogenic activity in their seminiferous tubules with many type B spermatogonia present in some sections but no primary spermatocytes. Two males collected on 7 January 1988 had numerous primary spermatocytes in their seminiferous tubules but no secondary spermatocytes. Spermatogenic activity was at a peak at the beginning of autumn (March and April) when masses of spermatozoa were released from the seminiferous tubules into the epididymides. Four males collected during March and three collected during April had large numbers of spermatozoa in both their caudae epididymides and seminiferous tubules (Table 1).

The testes of all males collected between May and September were spermatogenically inactive, and the seminiferous tubules were lined with Sertoli cells and occasional stem spermatogonia only. However, large numbers of spermatozoa were stored in the caudae epididymides throughout this period. Two of the three males collected on 6 November 1987 still had some spermatozoa in their caudae epididymides, although no sign of spermatozoa could be found in their seminiferous tubules. These spermatozoa were therefore left over from the previous cycle. It would therefore appear that spermatozoa were stored in the caudae epididymides for at least six months (May–October) after cessation of spermatogenic activity in the testes.

Copulations were initiated during late March and early April, and all females collected from the end of March to

Table 1 Sample sizes and reproductive conditions of monthly samples of male and female Cape serotine bats

Date	Sample size		Spermatozoa in			Leucocytes
	Males	Females	Testes	Epididymides	Uterus	in uterus
Jan '88	2	2	–	–	–	–
Feb '88	0	0				
Mar '88	4	2	+	+	+	+
Apr '88	1	4	+	+	+	+
'90	2	1	+	+	+	+
May '88	1	3	–	+	–	–
'90	3	2	–	+	+	+
Jun '90	0	0				
Jul '88	1	2	–	+	+	+
'90	1	2	–	+	+	+
Aug '88	4	7	–	+	–	–
Sep '89	5	5	–	+	–	–
Oct '83	0	5			–	–
'84	0	2			–	–
Nov '83	0	3			–	–
'87	3	0	–	+		

Spermatozoa: – = absent, + = present.
Leucocytes: – = absent, + = present.

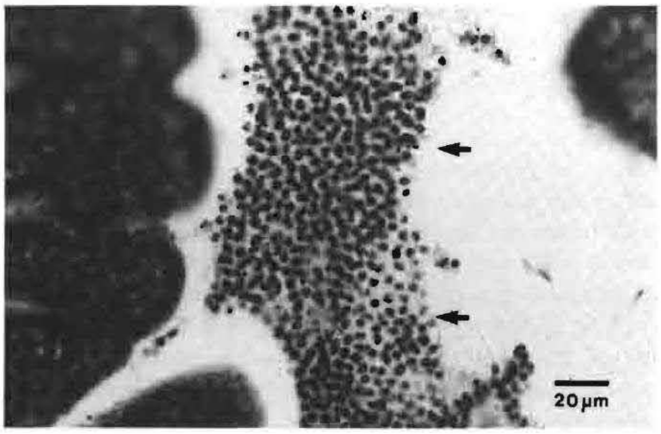

Figure 1 Leucocytes (arrows) in the uterine lumen of a female Cape serotine bat collected during May 1988.

late July had some spermatozoa in the proximal ends of their uterine horns (Table 1). During the same period all the females examined also had polymorphonuclear leucocytes in their uterine lumina and uterine glands (Figure 1, Table 1). No leucocytes, however, were found in the uterotubal junction area (UTJ) or oviducts. During March and April, when copulations began, leucocyte numbers were low compared to May when large numbers of leucocytes were present. Females collected at the end of July had relatively few leucocytes in their uterine horns.

Spermatozoa found in the uterine lumen of all females examined were not orientated in any specific manner, and showed no relationship with the epithelial lining of the uterine horns. No specific orientation of the spermatozoa could be found in the UTJ either, with the exception of one female collected during May in which the spermatozoa in the colliculus tubaricus part of the UTJ (see Mori & Uchida

Table 2 Number and developmental stage of conceptuses

Month	Female	Embryos/ foetuses	Development stage of embryos/mass (g) of foetuses
Aug '88	1	3	Morulae
	2	3	Morulae
	3	2	Young blastocysts (implanting)
	4	2	Morula + young blastocysts
	5	2	Young blastocysts
	6	2	Morulae
	7	3	Morulae
Sep '89	1	2	0,0243 + 0,0224
	2	2	0,0183 + 0,0134
	3	2	0,0107 + 0,0164
	4	3	0,0273 + 0,0271 + 0,0168
	5	4	0,0109 + 0,0113 + 0,0107 + 0,0093
Oct '83	1	2	0,1245 + 0,1055
	2	2	0,1164 + 0,0978
	3	2 (near term)	0,9800 + 0,8277
	4	2 (full term)	1,6494 + 1,4816

1980) were orientated with their heads towards the epithelial lining, with many resting against it. The spermatozoa in the intramural part of the UTJ were, as in all the other females examined, not orientated in any specific manner.

Ovulation occurred during the second half of August. All four females collected during July (29th 1988 and 31st 1990; Table 1) had mature Graafian follicles in their ovaries. However, all seven females collected during August (24th 1990; Table 2) had small embryos at various stages of development. The majority were morulae in the uterine lumen, with the oldest one being a young implanting blastocyst (Table 2). Five females collected during September all had small foetuses at the limb bud stage (Table 2).

Most gravid uteri contained two conceptuses (one in each horn), although singletons, triplets and even quadruplets were noted.

Four of five females collected at the end of October had large foetuses while one had already given birth and was lactating. One of the gravid females had two full-term foetuses that were fully pigmented and near birth (Table 2). The combined mass of the two foetuses was 3,13 g, comprising 43% of the average body mass of non-gravid females. Three females collected at the end of November were all lactating. Since parturition probably occurred from the end of October to mid-November and the majority of ovulations occurred during the second half of August, the gestation period is estimated to be about 12 weeks.

Discussion

The majority of southern African insectivorous bats so far investigated are monoestrous with one seasonal oestrous cycle per annum (see Rautenbach 1982; Skinner & Smithers 1990 for reviews). This appears to be the norm for most insectivorous bats (Wimsatt 1975; Hill & Smith 1984). However, recent evidence indicates that some South African bats deviate from this pattern. The little free-tailed bat, *Tadarida pumila*, is polyoestrous with up to three pregnancies per breeding cycle (Van der Merwe, Rautenbach & Van der Colf 1986). This is facilitated by a post-partum oestrus (Van der Merwe, Giddings & Rautenbach 1987). Recently it has been shown that twins are common amongst certain southern African bat species, and occasionally even triplets have been documented, viz. the yellow house bat *Scotophilus dinganii* (Rautenbach 1982), the lesser yellow house bat *Scotophilus borbonicus* (Van der Merwe, Rautenbach & Penzhorn 1988), and Schlieffen's bat *Nycticeius schlieffenii* (Van der Merwe & Rautenbach 1986). Although twins are common in the Cape serotine bat, singletons, triplets and even quadruplets (Lynch 1989; Van der Merwe 1990; present study) have been recorded for this species.

Vespertilionid and rhinolophid bats inhabiting temperate latitudes undergo spermatogenesis during summer, and mating is initiated during autumn (Racey 1982). Spermatozoa are stored in the female reproductive tract and retain their fertilizing capacity until spring, when ovulation occurs and the females become pregnant (Wimsatt 1969; Racey 1979). Male bats also store spermatozoa for prolonged periods, even after the females have ovulated (Racey 1973, 1979). It seems that the responsibility for sperm storage is shared between the sexes, with different sexes taking greater or lesser shares, depending on the species (Racey 1982). Racey (1979) mentioned that spermatozoa can be stored in the female tract for up to seven months, and may remain in the caudae epididymides of males for up to ten months. In South Africa sperm storage has also been recorded in vespertilionids (Bernard 1982; Van der Merwe & Rautenbach 1987, 1990) and in rhinolophids (Bernard 1983).

In the Cape serotine bat the testes become completely involuted and inactive during May, with spermatozoa being stored in the caudae epididymides at least until October. In this species spermatozoa are still present in the caudae epididymides for about two months after fertilization has occurred. In the northern Transvaal bushveld, Cape serotine bats are frequently active during winter and do not appear to go into prolonged periods of torpor, as evidenced by the fact that bats were taken during winter. This is not a strange phenomenon, as bat activity during winter has been recorded for much colder climates. In England, Ransome (1968, 1971) found that the greater horseshoe bat *Rhinolophus ferrumequinum* arouses to feed regularly during the winter hibernation period, and Racey (1982) mentioned that although there is evidence that repeated arousal and frequent activity during hibernation may be inimical to the storage of spermatozoa, it is clear that bats are often naturally active for a considerable part of the period of sperm storage.

In most mammals, including bats, there is a massive accumulation of leucocytes in the uterus shortly after insemination occurs (Hill & Smith 1984). However, in those bat species that store spermatozoa in the female tract, the reproductive tracts are remarkably free of leucocytes (Racey 1979) and this onslaught does not occur (Hill & Smith 1984). The presence of both spermatozoa and leucocytes in the uterine lumina and glands of female Cape serotine bats collected at different times during the winter suggests that spermatozoa are not stored in the uterine lumen. The sites of sperm storage in female bats vary with species and can be either the oviduct, UTJ or the uterus (see Racey 1979). According to Racey (1979) it is not always easy to decide which part of the tract fulfils this function, but that useful criteria are the identification of special relations, such as the attachment between spermatozoa and their storage organs or the presence of healthy spermatozoa. Although spermatozoa in the UTJ of the majority of Cape serotine bats examined were not orientated in any specific manner, no leucocytes were noted in this area, and it would therefore appear that this is the area where spermatozoa are present at least for some period of time. Whether spermatozoa in the UTJ are stored there from the onset of copulations (late March/early April) until fertilization (August) is doubtful. The fact that specific orientation of spermatozoa in the UTJ was observed in only one female, as well as the leucocytic activity found in the uterine lumina of the majority of females, even as late as 31 July, place a shadow of doubt on the females ability to store spermatozoa for prolonged periods. It is expected that leucocytes should be capable of removing all spermatozoa from the initial copulations (late March/early April) within the four-month period early April to the end of July. This argument is supported by the fact that the volume of spermatozoa found in the proximal parts of the uterine horns of this species, at any stage of the study, was absolutely minute when compared with that found in the coexisting rusty bat. In the latter species, the uterus is massively distended with semen (Van der Merwe & Rautenbach 1990). Therefore, the presence of spermatozoa and leucocytes in the uterine lumina of females collected even as late as 31 July would rather suggest more recent copulations with fresh leucocyte activity. Furthermore, as no conspicuous decrease in sperm volume was noted in the uterine lumina as the winter progressed, it would rather appear that the burden of prolonged sperm storage in this species falls on the male, and that periodic winter copulations combined with leucocytic activity, prevented a significant increase or decrease in sperm volume. In the Northern Hemisphere copulations during winter are well-documented in bats (Gilbert & Stebbings 1958; Wimsatt 1945). In South Africa (Transvaal Province), Van der Merwe & Rautenbach (1987) argued that there is a possibility that subsequent matings during winter may occur in the vespertilionid *Nycticeius schlieffenii*. However, in the vespertilionid *Pipistrellus rusticus*, which coexists with the Cape serotine bat, the absence of leucocyte activity and frequent copulations during winter are the result of a three-fold increase in sperm volume between April and July (Van der Merwe & Rautenbach 1990).

At present detailed knowledge regarding reproduction in South African vespertilionids is restricted to only a few species. Nevertheless, in these few species three different reproductive delay phenomena have been found, which to a greater or lesser extent overlap with the winter months. In Schreiber's long-fingered bat *Miniopterus schreibersii* (Bernard 1980; Van der Merwe 1980) and the lesser long-fingered bat *M. fraterculus* (Bernard 1980) delayed implantation occurs, lasting approximately four months in Schreiber's long-fingered bat, both in the Natal Midlands (Bernard 1980) and the Transvaal Highveld (Van der Merwe 1980).

Sperm storage or delayed ovulation has been described for Schlieffen's bat *N. schlieffenii* (Van der Merwe & Rautenbach 1987), the rusty bat *P. rusticus* (Van der Merwe

& Rautenbach 1990) and the Temminck's hairy bat *Myotis tricolor* (Bernard 1982). A third delay phenomenon described for the lesser yellow house bat *Scotophilus borbonicus* is retarded embryonic development after implantation (Van der Merwe *et al.* 1988).

Both Schlieffen's bat and the lesser yellow house bat coexist at *ca.* 29°S in the northern Kruger National Park. In both species a reproductive delay of approximately three months occurs. In the lesser yellow house bat the delay is caused by retarded embryonic development which occurs directly after implantation and lasts from April/early May to the end of July (Van der Merwe *et al.* 1988). In Schlieffen's bat the delay is caused by the storage of spermatozoa, in both sexes, from June to the end August (Van der Merwe 1987). In both species the young are born during November.

Despite differences during the delay period, such as sperm volume, sperm storage sites and the presence of leucocytes, the reproductive cycles of the rusty bat (Van der Merwe & Rautenbach 1990) and the Cape serotine bat (present study) coexisting in the northern Transvaal bushveld at *ca.* 23°S are very similar. In both species spermatozoa are present simultaneously in the caudae epididymides of the male and genital tract of the female for approximately five months of the year (April–August) before ovulations and fertilization occur. In both species the majority of births occur during November.

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