Monthly changes in the female reproductive organs and the reproductive cycle of *Myotis tricolor* (Vespertilionidae : Chiroptera)

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Myotis tricolor was monoestrous and monotocous. The oestrous cycle was characterized by a prolonged period of oestrus between mid-April and mid-September and a short period of anoestrus between the termination of lactation and the onset of pro-oestrus. The female reproductive cycle was characterized by copulation in mid-April and ovulation and fertilization in mid-September. The gestation period was estimated to have been 63 days and parturition occurred between mid-November and mid-December. Parturition was followed by a six-week period of lactation. It is assumed that between mid-April and mid-September the female stored spermatozoa in the uterine horns, a reproductive strategy previously reported for north-temperate members of this genus.

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Maandelikse veranderinge in die vroulike voortplantingsorgane van *Myotis tricolor* is gekenmerk deur 'n kort ontwikkelingsperiode van die follikels van Graaf sowel as die vaginale epiteel gedurende pro-estrus. Die estrussiklus is gekenmerk deur 'n uitgerekte estrus en 'n kort anestrus tussen die einde van laktasie en die begin van pro-estrus. Die vroulike voortplantingsiklus is gekenmerk deur kopulasie in middel April, ovulasie en bevrugting in middel September en geboorte tussen middel November en middel Desember. Dit word vermoed dat spermatozoa tussen middel April en middel September in die uterushorings gestoor word, 'n voortplantingstrategie reeds voorgestel vir noord-gematigde lede van hierdie genus.

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The genus *Myotis* is the most widely distributed of all groups of bats, being absent only from the arctic, subarctic, and antarctic regions (Walker 1964). In the tropics members of this genus are polyoestrous (Dwyer 1970; Wilson & Findley 1970; Myers 1977) while in temperate latitudes sperm storage occurs during winter hibernation (Guthrie 1933; Kunz 1973; Oxberry 1979). In areas of intermediate latitude and/or climate, polyoestry occurs in association with sperm storage (Myers 1977). Polytocy, both by itself and in association with sperm storage, has been described in *M. austroriparius* (Rice 1957).

In Natal, aspects of reproduction of *M. tricolor* have been described by Harrison & Clancey (1952) and Laycock (1976). Laycock (1976) noted that *M. tricolor* is absent from the study area during winter and that only females are present during summer, forming maternity colonies. This absence from the study area between mid-April and late September has resulted in several questions remaining unanswered. The aim of the present study was to investigate the reproductive and oestrous cycles of *M. tricolor* and to see if and how they have been modified when compared with the cycles of tropical members of this genus.

Materials and Methods

Specimens of *M. tricolor* were collected from several roosts (caves and disused mines) in the Natal Midlands (*c.* 29°S) on an approximately two-weekly basis during 1977 and 1979. Specimens were killed by asphyxiation with carbon dioxide and the female reproductive tract removed under a dissecting microscope. Tissues were fixed in Bouin's fluid and stored in 70% alcohol. Following routine embedding and sectioning at 5 μm, sections were stained with Erlich's haematoxylin and eosin.

All microscopic measurements were made with an optical micrometer. Ovarian activity was quantified by measuring the diameter of all secondary and Graafian follicles and plotting mean monthly diameters for these follicle types. Mean follicular diameter was calculated from two measurements taken at right angles to each other, one of which was always the largest diameter. Monthly changes in the vagina were quantified by measuring thickness of the vaginal epithelium and superficial cornified layer, when present, in approximately 10 positions per specimen and plotting mean monthly thickness. Monthly changes in the uterine horn wall were

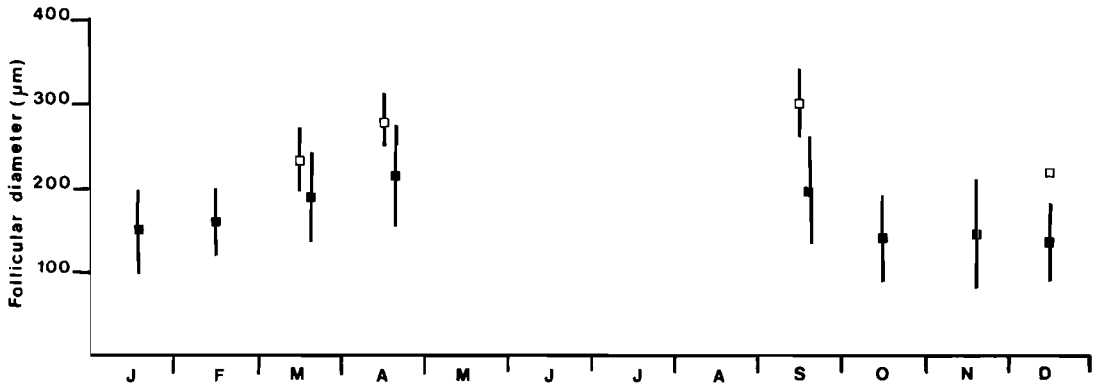

Figure 1 Monthly changes in secondary and Graafian follicle diameter. Open squares indicate mean monthly Graafian follicle diameter and solid squares mean monthly secondary follicle diameter. Vertical lines indicate $\bar{X} \pm 2SD$.

quantified by measuring thickness of the uterine horn wall and endometrium in about 10 positions per specimen and plotting mean monthly thickness.

It has been shown (Richardson 1977) that when studying the reproductive cycle, in Microchiroptera in particular, conclusions based on observations of changes in gross morphology of the female reproductive tract and changing mass of the testes and epididymides can be deceptive. For this reason the present study has been based on a histological examination of ovaries, uterine horns, and vagina. Throughout this report, where sample size was more than 10, mean values have been given plus or minus two standard deviations. Where sample size was less than 10 the mean value alone has been given.

Results

Monthly changes in the ovary (Figure 1)

No significant difference was found in mean monthly follicular diameters between the two ovaries and in Figure 1 measurements from the two ovaries have been pooled. Between December and February the ovary appeared to be in a resting condition with little development of secondary follicles. During this period the cortex contained large amounts of hypertrophied interstitial gland cells and only a few secondary follicles.

During March and April there was a period of follicular development during which secondary follicle diameter increased from 160,5 ± 37,2 μm in February to 210,8 ± 40,7 μm in April. In late March the first Graafian follicles were seen and by 11 April, when the last specimens were collected before the species left the study area, Graafian follicle diameter had reached 276,3 ± 33,0 μm. When the species returned to the study area in late September ovulation and fertilization had recently occurred and there was a single corpus luteum in one of the ovaries. The diameter of the corpus luteum increased up to the time of embryonic implantation and decreased towards parturition (Table 1). The cells of the corpus lutuem underwent changes in size that followed those of the corpus lutuem (Table 1).

Between September and early December there were a large number of developing secondary follicles in the ovaries and while most of these underwent atresia before becoming vesicular a few follicles reached this stage before atresia occurred.

The cells of the interstitial gland tissue in the cortex existed in two forms; a hypertrophied and presumably active form, and a smaller, inactive form. The interstitial cells were in the hypertrophied form during September and during lactation, and anoestrus between December and February. Between these two periods the cells reverted to their normal form and maintained a more or less constant diameter of 10,3 μm (Table 2).

Monthly changes in the vagina (Figure 2)

Between December and February the vaginal epithelium, which comprised three to four layers of nucleated cells, appeared to be in a resting stage. There were no cell divi-

Table 1 Changes in corpus diameter and luteal cell diameter arranged according to the stage of pregnancy of the specimen

Stage of pregnancy	Date	No.	Mean corpus luteum diameter (μm)	Mean luteal cell diameter (μm)
Conceptus in right oviduct	20/9	1	382,4	10,9
16-cell morula in right uterine horn	20/9	1	437,9	11,6
32-cell morula in right uterine horn	20/9	1	581,4	16,6
Late implantation	28/9 & 1/10	2	694,5	18,1
		2		
Developing foetus	9/10 – 20/11	12	504,0	14,3
Lactating	25/11 & 13/12	1	180,2	11,2
		3		

sions in the stratum germinativum and the epithelium maintained a more or less constant thickness. During March and April there was an increase in epithelial thickness from 22,3 ± 8,6 μm in February to 45,9 ± 10,7 μm in April. During March the first superficial layers of cornified cells were present and in specimens collected on 11 April this layer had reached a thickness of 14,6 ± 9,8 μm. Copulation had occurred in the specimens collected on 11 April and delamination of the cornified layer was occurring.

Table 2 Mean monthly interstitial gland cell diameter. Note the increased cell size in September and between December and February

Months	Mean cellular diameter (μm)
January	13,1 ± 3,4
February	12,2 ± 1,5
March	10,5 ± 1,6
April	10,3 ± 3,2
September	12,3 ± 3,0
October	10,1 ± 4,8
November	10,2 ± 2,8
December	12,8 ± 2,2

In late September, when the species returned to the study area, the vaginal epithelium consisted of between six and eight layers of nucleated cells. The cells of the outer layers were squamous-like and were being sloughed off. Between September and December a constant but slight delamination resulted in a slow decrease in epithelial thickness. During this period the epithelium comprised nucleated cells and scattered leucocytes.

Monthly changes in the uterine horn (Figures 3 and 4)

Between late December and early March the endometrium maintained a more or less constant thickness and the uterine glands were short and straight. During March and April there was a twofold increase in endometrial thickness of both uterine horns and the uterine glands increased in length.

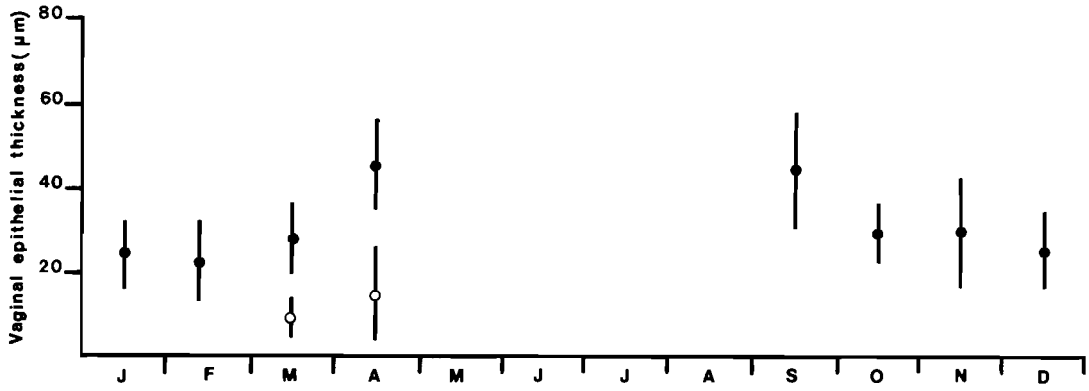

Figure 2 Monthly changes in thickness of vaginal epithelium. Solid circles indicate mean monthly thickness of the vaginal epithelium, and open circles mean monthly thickness of cornified layer. Vertical lines indicate $\bar{X}$ ± 2SD.

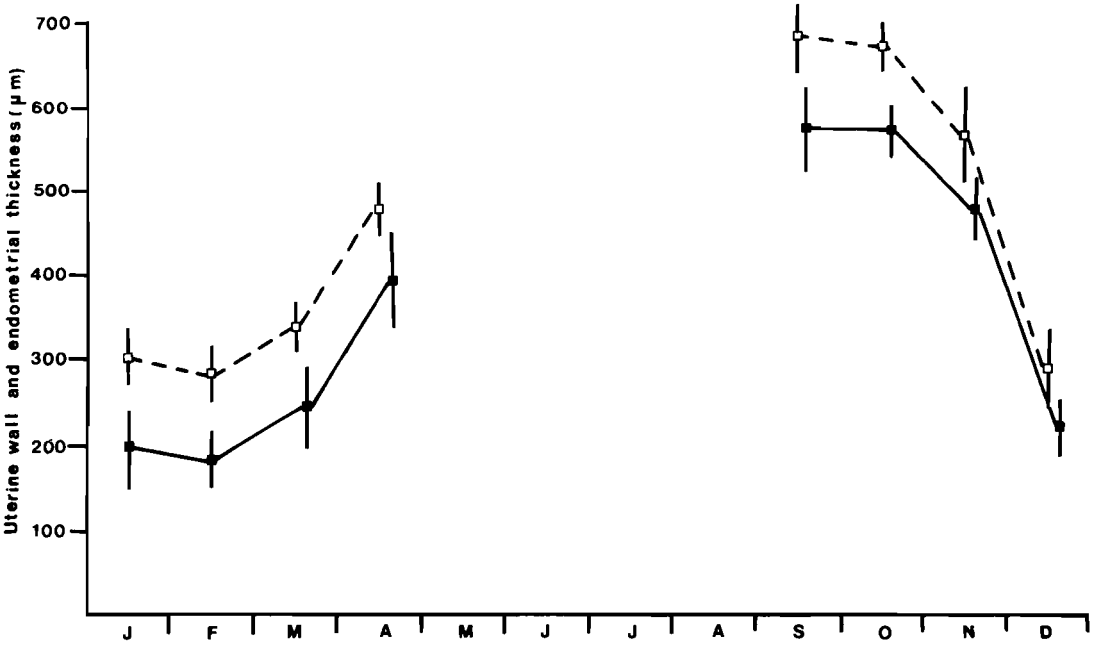

Figure 3 Monthly changes in thickness of right uterine horn. Open symbols indicate mean monthly thickness of uterine horn wall and solid symbols mean monthly thickness of the endometrium. Vertical lines indicate $\bar{X}$ ± 2SD.

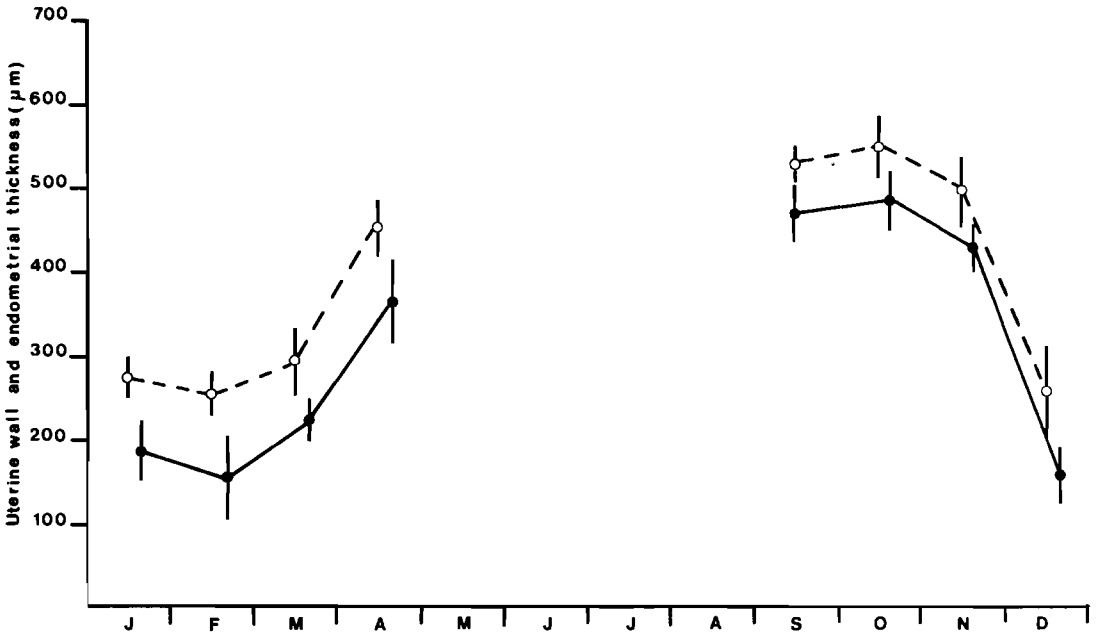

Figure 4 Monthly changes in thickness of the left uterine horn. Open symbols indicate mean monthly thickness of the uterine horn wall, and solid symbols mean monthly thickness of the endometrium. Vertical lines indicate $\bar{X} \pm 2SD$.

When the species returned to the study area ovulation and fertilization had occurred and the endometrium was in a typical progestational condition; the uterine glands were long, coiled, and branched, the lumina of the glands were enlarged, and the sub-mucosa was highly vascularized.

During late November and December, in lactating specimens, breakdown of the endometrium was occurring and the lumina of the uterine horns were filled with cellular debris and leucocytes.

Spermatozoa were first seen in the lumina of the corpus of the uterus, uterine horns, and uterine glands of specimens collected on 11 April and were absent from specimens collected on 20 September. In the uterine horns and uterine glands the spermatozoa were arranged so that their heads were in contact with the uterine epithelium. Elsewhere in the female reproductive tract the spermatozoa were haphazardly arranged.

Oestrous cycle of *Myotis tricolor*

Although *M. tricolor* was absent from the study area for four months all five stages of the oestrous cycle were observed. Pro-oestrus occurred between late February and early April when there were developing secondary and Graafian follicles in the ovaries and the vaginal epithelium and uterine endometrium were undergoing a period of rapid development.

Two specimens collected on 11 April were in oestrus. Although the ovaries did not contain preovulatory Graafian follicles the vaginal epithelium had an outer layer of cornified cells and copulation had occurred.

When specimens returned to the study area in late September ovulation and fertilization had recently occurred, suggesting that these bats had been in oestrus or submaximal oestrus throughout their absence. One of the specimens collected on 20 September was in metoestrus; there was a four-cell conceptus in the right oviduct and delamination of the vaginal epithelium was occurring. Two other specimens collected on 20 September, all specimens collected in October and the first half of November, and some specimens from late November and early December were in the pregnancy/luteal stage. In these specimens there was no development of Graafian follicles and the vaginal epithelium was undeveloped. Parturition occurred between mid-November and mid-December and was followed by a period of lactation anoestrus. The last lactating bat was collected in late January, approximately six weeks after the end of parturition. Lactation anoestrus was characterized by the almost complete absence of Graafian follicles from the ovaries, an undeveloped vaginal epithelium, and breakdown of the endometrium. Although lactation lasted only until January, the anoestrous condition continued until the end of February.

Gestation period of *Myotis tricolor*

The length of the gestation period was estimated from specimens collected from Otto's Bluff cave during 1979. Fertilization had recently occurred in three specimens collected on 20 September (one contained a four-cell conceptus in the left oviduct and two contained individual morulae in the right uterine horn) and parturition was first observed to have occurred in one specimen collected on 17 November. This is a period of 58 days. Assuming that fertilization had occurred approximately five days before the first specimens were collected then the gestation period is estimated to have been 63 days.

Discussion

Monthly changes in the reproductive organs and the oestrous cycle of *Myotis tricolor* were typically mammalian and generally similar to those reported for other vespertilionids in which sperm storage occurs (Wimsatt 1944, for *M. lucifugus*; Kitchener 1975, for *Chalinolobus gouldii*; Myers 1977, for *Myotis albescens*; and Kitchener & Halse 1978, for *Eptesicus regulus*, and summarized by Oxberry 1979).

The cyclical change in size that the corpus luteum of

M. tricolor underwent (increasing up to the time of implantation and decreasing towards parturition) has been reported in *Lasiurus ega, Eptesicus furinalis, Myotis albescens, M. nigricans* (Myers 1977) and *Miniopterus schreibersi* (Bernard 1980b) but differs from that reported in *Pipistrellus pipistrellus* where corpus luteum diameter reaches its maximum approximately two weeks before parturition (Racey & Swift 1981). In *Miniopterus schreibersi* it has been shown that the placental discs may be important sites for the production of oestrogen and progesterone (Fonda & Peyre 1965; Peyre & Malassine 1969) and since, in *M. schreibersi*, the decrease in corpus luteum diameter coincides with formation of the placenta (Bernard 1980a), it has been suggested that, as in other mammals, the placenta may take over part of the role of the corpus luteum after implantation. For the same reason it is suggested that the decrease in corpus luteum diameter in *Myotis tricolor* after implantation may indicate that the placenta takes over part of its role.

The ovary of *M. tricolor* contained large amounts of interstitial gland tissue, the prime source of which appeared to be the theca folliculi of atretic follicles (Bernard 1980a). Hypertrophy of the interstitial gland cells, which occurred during lactation in *M. tricolor*, has been reported also in *M. lucifugus* (Guthrie & Jeffers 1938). This hypertrophy may have been a result of the increased number of atretic follicles observed during November and December (Bernard 1980a). Interstitial gland cells are endocrine in function (Mossman & Duke 1973) and it is assumed that the increase in interstitial gland cell size during lactation indicates that these cells were possibly associated with the hormonal control of lactation. The significance of the appearance of hypertrophied interstitial cells during September is not known and the absence of specimens for the preceding months prevents valid conclusions from being drawn.

Three basic reproductive strategies have been reported for the genus *Myotis*, these being polyoestry, polytocy, and sperm storage with associated delayed ovulation.

Myotis nigricans from Barro Colorado Island (*c.* 9°N) (Wilson & Findley 1970) and Paraguay (*c.* 24°S) (Myers 1977), and *M. adversus* from southeastern and east-central Queensland (*c.* 27°S and *c.* 22°S) (Dwyer 1970) are seasonally polyoestrous and monotocous, producing between two and three young per year. The gestation period of *M. nigricans* (*c.* 9°N) is between 50 and 90 days while that of *M. nigricans* (*c.* 24°S) and *M. adversus* (*c.* 27°S and 22°S) is about 70 days (Myers 1977 and Dwyer 1970 respectively).

M. albescens from Paraguay (*c.* 24°S) is polyoestrous but, between late May and early August, in one of the oestrous cycles there is a period of sperm storage (Myers 1977). The length of the gestation period of *M. albescens* is about 90 days (Myers 1977).

M. austroriparius from Florida (*c.* 28°N) is monoestrous and polytocous producing two young per pregnancy (Rice 1957). The same species from *c.* 32°N in Florida is also monoestrous and polytocous but includes a period of sperm storage during winter (Rice 1957).

M. lucifugus and *M. grisescens* from *c.* 39°N (Guthrie 1933) and *M. velifer* from *c.* 37°N (Kunz 1973) are monoestrous, monotocous, seasonal breeders in which sperm storage and delayed ovulation occur. The gestation period of these species is between 60 and 70 days.

It would appear therefore that the typical reproductive strategy in the tropics is polyoestry while that in temperate latitudes is monoestry associated with a period of sperm storage during winter hibernation. Polytocy has been reported from only *M. austroriparius*, a monoestrous species, and it is possible that polytocy is an adaptation to maintain a birth rate of two young per year in an environment that does not permit polyoestry.

In areas intermediate between tropical and temperate climates polyoestry and polytocy are associated with a period of sperm storage during a period of reduced environmental temperature (Rice 1954; Myers 1977). Although sperm storage is in most cases associated with winter hibernation (Guthrie 1933; Kunz 1973; Oxberry 1979) this is not simply a cause and effect relationship. Short periods of sperm storage have been reported in non-hibernating Microchiroptera (Jerret 1979) and Myers (1977) reported a three-month period of sperm storage during winter in *M. albescens*, a species which does not hibernate in Paraguay.

The facts available indicate that in *M. tricolor* copulation occurred in April, shortly before the species left the study area, and that ovulation and fertilization, as indicated by the presence of early developmental stages in the oviducts and uterine horns, probably occurred in mid-September, shortly before the species returned to the study area. These data suggest that sperm storage probably occurred during winter and ovulation was delayed until spring, thus conforming to the pattern established in temperate members of the genus *Myotis*. Assuming that sperm storage did occur in *M. tricolor*, then its oestrous cycle was characterized by a long period of oestrus or submaximal oestrus between April and September and a short period of anoestrus between the termination of lactation and onset of pro-oestrus.

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