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Cycle of the Seminiferous Epithelium in the Grey-headed Fruit Bat, *Pteropus poliocephalus*

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Abstract

The seminiferous epithelial cycle of wild *P. poliocephalus* could be divided into eight stages on the basis of cellular associations and nuclear morphology. The relative frequencies of the stages (1–8) were, respectively: 15·8, 20·5, 9·4, 8·9, 11·1, 7·7, 9·8, and 16·8%. The duration of the cycle was determined by intratesticular injection of [^{3}H]thymidine followed by autoradiography and estimated to be 16·0 days. The duration of meiotic prophase and spermiogenesis were both 23·3 days. Spermatocyte DNA synthesis appeared to occur in leptotene primary spermatocytes in stage 2. The duration of spermatogenesis is similar to that in man although the organization of the cycle resembles that of rodents.

Introduction

The grey-headed fruit bat, *Pteropus poliocephalus*, is a short-day breeder which inhabits the coastal regions of eastern Australia (Ratcliffe 1931). Adult males show seasonal changes in testis size which are maximal during autumn just prior to the time of mating (Nelson 1963, 1965). The cycle of the seminiferous epithelium has been analysed in many mammalian species, including the rat (Roosen-Runge and Giesel 1950), rabbit (Swiestra and Foote 1963), boar (Swiestra 1968), mouse (Oakberg 1956), monkey (Clermont and Leblond 1959), man (Heller and Clermont 1963), vole (Grocock and Clarke 1975), nine-banded armadillo (Torres *et al.* 1981) and a microchiropteran bat, *Rhinopoma kinneari* (Singwi and Lall 1983) but little information is available on the cycle of the seminiferous epithelium in the Chiroptera as a whole and in the Megachiroptera in particular. The phylogenetic relationship between the Mega- and Microchiroptera is under question; Pettigrew (1986) suggests that megachiropterans could be prosimian primates. The present study provides a detailed analysis of the seminiferous epithelial cycle in the fruit bat, including an estimate of its duration.

Materials and Methods

General

Adult male *P. poliocephalus* were obtained throughout the year from camps in the south-east Queensland region either by hand-capture or by shooting. Captured animals were killed by barbiturate overdose within a few hours of capture. Reproductive organs were removed immediately after death and either directly immersed in Susa's fixative or left on ice for less than 2 h prior to fixation.

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Histology

Tissue was fixed for 24 h, stored in 70% (v/v) ethanol, dehydrated in a graded series of alcohols, cleared in toluene, and infiltrated and embedded in Paraplast (Medos). Testis cross-sections 5μm thick were stained with Mayer's haematoxylin and alcoholic eosin after treatment with Lugol's iodine and sodium thiosulfate solutions to remove any remaining mercuric chloride crystals (Humason 1972).

Analysis of Spermatogenesis

The seminiferous epithelial cycle was divided into stages according to the cellular associations and nuclear morphology apparent in circular cross-sections of seminiferous tubules (Roosen-Runge and Giesel 1950). The relative frequency of each stage was determined by classification of 1500 circular seminiferous tubule cross-sections from 10 testes collected during the breeding season. An estimate of the duration of spermatogenesis was made by using [^{3}H]thymidine administration and autoradiography (Clermont *et al.* 1959). Two adult males captured during April were anaesthetized with 20 mg ketamine and injected directly into each testis with 1·85 mBq [^{3}H]methyl thymidine (Amersham Australia) prepared by mixing 0·05 ml sterile saline with 0·05 ml [^{3}H]thymidine (37 mBq/ml aqueous). Testes were then removed, either by hemicastration or after death 80 min, 73 h, 168 h and 502 h after thymidine administration. Testes were fixed for 24 h in Bouin's fixative, processed and sectioned as before with the exception of the deletion of the mercuric chloride removal steps. Sections were dewaxed, hydrated and hand-dipped in a 1 : 1 mixture of Ilford K2 emulsion (Ilford Australia) and 2% (v/v) glycerol in water according to the recommended methods of Rogers (1979). Autoradiographs were then dried over silica gel and exposed for 3 weeks prior to development with Ilford PQ developer (Rogers 1979). Developed autoradiographs were stained lightly with haematoxylin and eosin and mounted in DePeX (Gurr). Labelled germ cells were classified according to type and stage and the most advanced labelled cell type was determined for each sampling time. Sampling times and position of the label, combined with the relative durations of the stages allowed several estimates of the duration of spermatogenesis to be made.

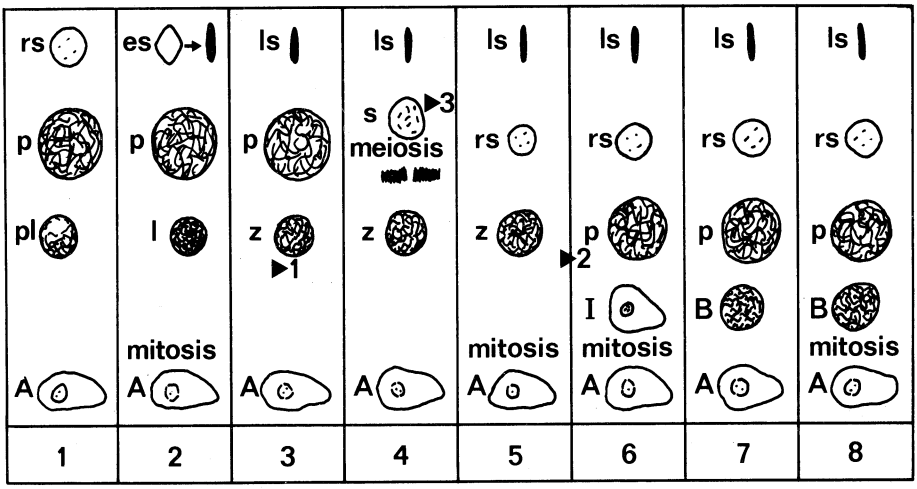


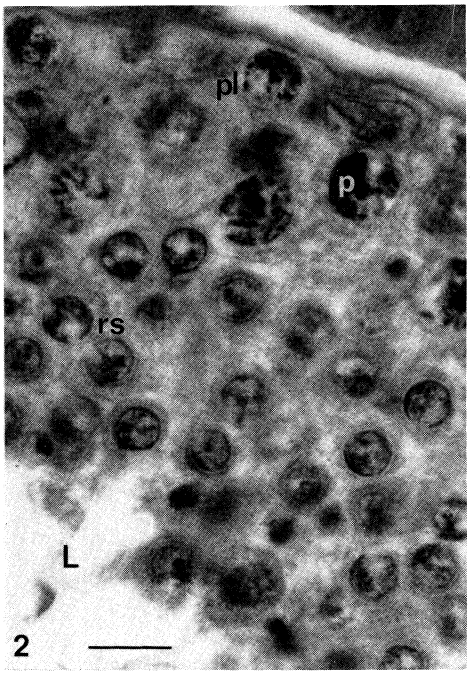
Fig. 1. Schematic representation of the eight stages of spermatogenesis in the fruit bat, as determined by cellular associations and nuclear morphology. Arrows (►) indicate the most advanced labelled cell type (►1 = 73, ►2 = 168·3, ►3 = 502 h) after [^{3}H]thymidine administration. *A*, A spermatogonia; *B*, B spermatogonia; *es*, elongating spermatids; *I*, intermediate spermatogonia; *l*, leptotene primary spermatocytes; *ls*, elongated spermatids; *p*, pachytene primary spermatocytes; *pl*, preleptotene primary spermatocytes; *rs*, round spermatids; *s*, secondary spermatocytes; *z*, zygotene primary spermatocytes.

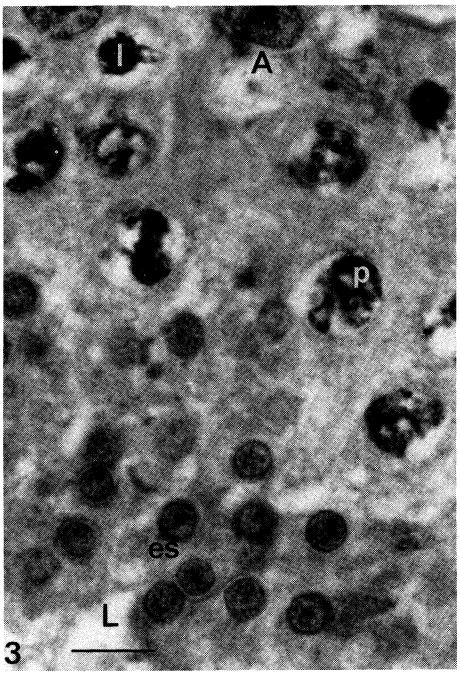
Results

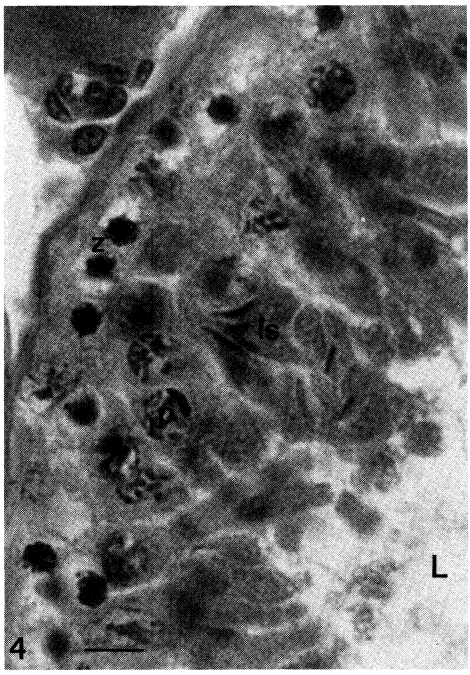
Stages of the Cycle of the Seminiferous Epithelium

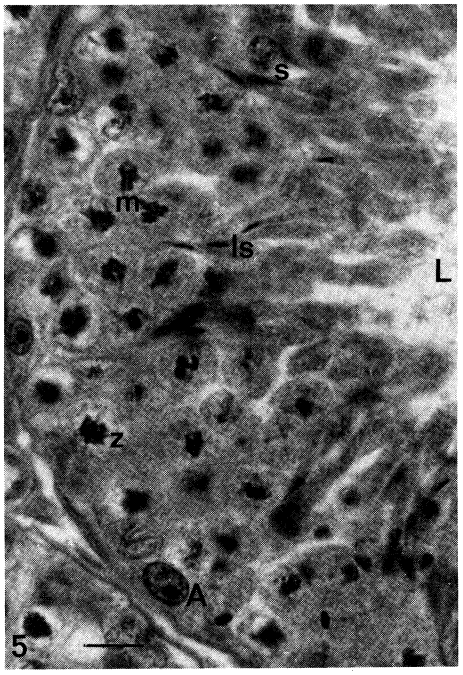
Spermatogenesis was divided into eight stages on the basis of cellular associations

and nuclear morphology. Fig. 1 is a schematic representation of the nuclear types present in each stage, Figs 2–9 are examples of each stage.

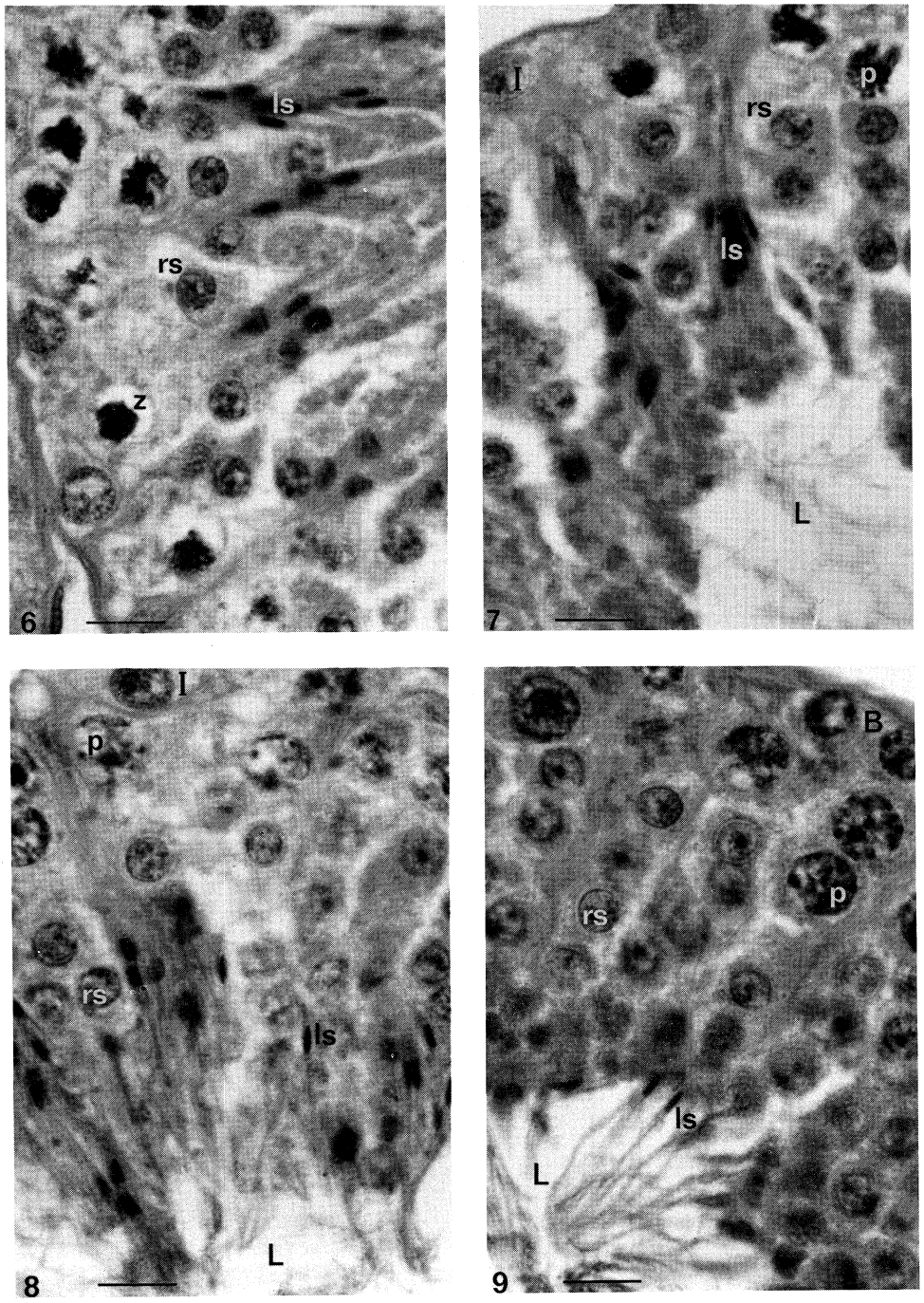








Figs 2–5. Stages 1–4, respectively, of spermatogenesis in the fruit bat classified by cellular associations and nuclear morphology. Abbreviations as for Fig. 1. *L*, tubular lumen. Haematoxylin and eosin. Scale bars = 10 μm.



Figs 6–9. Stages 5–8, respectively, of spermatogenesis in the fruit bat classified by cellular associations and nuclear morphology. Abbreviations as for Fig. 1. *L*, tubular lumen. Haematoxylin and eosin. Scale bars = 10 μm.

Stage 1. This stage occurred immediately after spermiation. The peripheral tubular layer consisted of type A spermatogonia and large numbers of preleptotene primary spermatocytes. Type A spermatogonia possessed ovoid nuclei with a small nucleolus but as type A spermatogonial subtypes were not easily discernible in this species, none were defined for the purpose of this study. Internal to the preleptotene nuclei were several layers of large pachytene primary spermatocytes which possessed spherical nuclei containing dispersed chromatin. Several layers of small spherical spermatid nuclei surrounded the tubular lumen which was either empty or contained some recently released spermatozoa. Residual bodies, appearing as dark bodies close to the lumen, were present early in stage 1.

Stage 2. Type A spermatogonia were present and mitosis was observed during this stage. The preleptotene primary spermatocytes had been replaced by a ring of leptotene primary spermatocytes with darkly staining nuclei, and the pachytene nuclei were unchanged. Spermatid nuclear elongation began concurrently with the appearance of the leptotene primary spermatocytes; as the spermatid nuclei elongated they formed groups so that at the end of this stage they appeared as clusters of condensed nuclei.

Stage 3. Type A spermatogonia were present but mitoses were not observed. The leptotene primary spermatocytes had been replaced by zygotene primary spermatocytes which exhibited nuclei with flaky chromatin. The pachytene primary spermatocytes were unchanged, lying external to the elongated spermatids which were clearly grouped in separate clusters around the tubule.

Stage 4. This stage was characterized by the occurrence of meiosis. Meiotic divisions did not occur completely synchronously in any tubular cross-section so that in this phase it was common to observe diplotene primary spermatocytes, meiotic divisions and secondary spermatocytes in the same tubular cross-section. No spermatogonial mitoses were observed, and the zygotene primary spermatocytes and elongated spermatids were unchanged.

Stage 5. Spermatogonial division was observed during this stage in which the zygotene nuclei were the only spermatocyte cells present. Round and elongated spermatids were present internal to the spermatocytes.

Stage 6. In addition to type A spermatogonia, intermediate type spermatogonia, which possessed more spherical nuclei and prominent nucleoli, were present and mitoses were observed. The zygotene primary spermatocytes had become pachytene primary spermatocytes which possessed a larger nucleus with more dispersed chromatin. The two generations of spermatids were unchanged in position and appearance.

Stage 7. The intermediate spermatogonia of stage 6 had been replaced by B spermatogonia which possessed spherical nuclei containing crust-like accumulations of chromatin and no obvious nucleoli. The pachytene primary spermatocytes and round spermatids were unchanged but the elongated spermatids were losing their groupings and moving toward the lumen.

Stage 8. B spermatogonia formed an almost continuous ring around the periphery of the tubule with a few type A spermatogonia. Spermatogonial mitosis was frequently observed during this stage. The pachytene primary spermatocytes and

round spermatids were unchanged. The long spermatids were at the luminal edge of the tubule and spermiation marked the end of this stage. Dark residual bodies were characteristic of this stage although they often persisted into the next stage (stage 1).

Duration of the Cycle of the Seminiferous Epithelium

The relative frequencies (durations) of the stages of the cycle are presented in Table 1. The stage with the longest relative duration was stage 2 which includes spermatid nuclear elongation. The most advanced points of radioactive labelling

Table 1. Stage frequencies and durations for the eight stages of spermatogenesis in the fruit bat (*Pteropus poliocephalus*)

Duration of the stages was calculated using the estimate for the duration of the cycle (Table 2) and the relative frequencies

Stage	1	2	3	4	5	6	7	8
Frequency (%)	15·8	20·5	9·4	8·9	11·1	7·7	9·8	16·8
Duration (days)	2·53	3·28	1·50	1·42	1·78	1·23	1·57	2·69

for the four sampling times after [^{3}H]thymidine administration are shown in Table 2 and Fig. 1. No labelling was detectable in the testis sampled 1·5 h after administration. Labelling was present in zygotene primary spermatocytes in stage 3 after 3 days (73 h). Labelling was present in zygotene primary spermatocytes becoming pachytene primary spermatocytes as stage 5 becomes stage 6 after 7 days (168 h); this was determined by analysis of transverse and longitudinal cross-sections.

Table 2. Estimates of the duration of the seminiferous epithelial cycle in the fruit bat (*Pteropus poliocephalus*)

Cell type abbreviations as in Fig. 1

Hours after [^{3}H]thymidine administration	Cell type	Cycle and stage	No. of cycles traversed	Interval (days)	Duration of cycle (days)
73	z	II; 3	–		
168	z/p	II; 5/6	0·247	3·97	16·1
502	s	III; 4	1·114	17·88	16·0
502 *v.* 168			0·867	13·90	16·0

Twenty-one days (502 h) after administration, labelling was present in secondary spermatocytes in the latter part of stage 4. Based on these three points of labelling and labelling times three estimates of the duration of the cycle have been made (Table 2). The three estimates show the duration of the cycle in the fruit bat to be 16 days.

Discussion

During the breeding season spermatogenesis in the fruit bat showed no peculiarities when compared with other mammals. Circular cross-sections typically showed only one discrete stage of spermatogenesis indicating that spermatogenesis occurs

in 'waves' along seminiferous tubules in this species. The cellular associations present did not differ greatly from those reported in the rat (Roosen-Runge and Giesel 1950). The only other member of the Chiroptera for which a detailed analysis of the seminiferous epithelial cycle is available is a non-scrotal microchiropteran, *Rhinopoma kinneari* (Singwi and Lall 1983). The cellular associations in the fruit bat more closely resemble the rat than those present in *R. kinneari*. This supports evidence which suggests that the two suborders of bats may be widely separated phylogenetically (Smith 1977). Distinct type A spermatogonial subtypes were not present in the fruit bat, making it difficult to reconstruct the early stages of spermatogenesis and assign a starting point (division giving rise to the stem spermatogonia) for the process. Presumably spermatogonial mitoses observed during stage 5 give rise to intermediate spermatogonia present in stage 6, the mitoses in stage 6 give rise to B spermatogonia in stage 7, and the mitoses in stage 8 give rise to the first generation of primary spermatocytes in stage 1. The mitoses observed in stage 2 must be a division of either stem spermatogonia or some later A spermatogonial subtype; this type of A spermatogonial division may also occur during stages 5, 6 and 8 concurrently and may be masked by the mitotic divisions described above. If the mitotic division in stage 2 is taken to be the beginning of spermatogenesis then this process in the fruit bat takes place over 3·84 cycles although, depending on the actual place of stem cell formation, this is probably an underestimate. The cycle of the fruit bat seminiferous epithelium has a longer duration than all mammalian species from which an estimate has been made with the exception of man which possesses a cycle of similar duration (Heller and Clermont 1963). Assuming spermatogenesis takes place over at least 3·84 cycles then the duration of spermatogenesis can be estimated to be greater than 60 days. Using this estimate of the duration all three points of labelling indicate that the initial thymidine uptake (DNA synthesis) occurred in leptotene primary spermatocytes in stage 2. The duration of both meiotic prophase and spermiogenesis are 23·3 days in the fruit bat as compared to 14·9 and 20·6 days, respectively, in the Sherman rat (Clermont *et al.* 1959) and 23·2 and 22·5–23·0 days, respectively in man (Heller and Clermont 1964).

In conclusion, the seminiferous epithelial cycle of the fruit bat has as its only distinctive feature a long duration which is similar only to that shown by man. The cellular associations present are not similar to man which exhibits several stages in single tubular cross-sections (Heller and Clermont 1964) but similar to that shown by rodents. Further work on spermatogenesis in this species includes investigation of seasonal changes in the dynamics of spermatogenesis.

Acknowledgment

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