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Chronology of Meiosis and Spermiogenesis of *Mabuyia carinata*.

P. K. Mallick[1], T. Banerjee[2], S. C. Maity[2] and S. K. Ghosal[2]

1. Department of Zoology, Dum Dum Motijheel College, 1, Motijheel Avenue, Kolkata – 700 074, West Bengal, India,
2. Molecular Genetics Lab., Department of Zoology Burdwan University-713 104, West Bengal, India.

^{3}H-thymidine autoradiography of *Mabuyia carinata* spermatocytes monitored sequentially revealed that the zygotene, diplotene, diakinesis and metaphase stages is remarkably shorter (being individually hardly a day) in contrast to prolonged pachytene (1.92 day), leptotene (1.40 day) and spermiogenesis (9.00 day). Radioactive spermatozpa appear for the first time at 14.52 d.p.i. Phylogenetically the total duration increases from fish to mammals as regards meiosis and spermiogenesis.

Key Wards : Gamatogenesis, Reproductive physiology, Chronobiology, Spermatogenesis.

Introduction :

The Leblond school of workers at McGill University, Montreal pioneered not only the stages of the cycle of the seminiferous epithelium in mammals (Leblond and Clermont, 1952, Clermont *et. al*., 1959) but also the duration of individual stages of meiosis and spermiogenesis in these animals (Clermont and Harvey, 1965). Attention was shortly diverted from rodents and legomorpha to man, monkey, ram and other animals (Clermont and Leblond, 1955, Heller and Clermont, 1963). One of us (SKG) conducted the work on the spermatogenesis chronology of golden hamster and mouse (Ghosal and Mukherjee, 1971, 1977) in the same Institution. Although several vertebrates covering fish (Sinha *et. al*. 1979, 1982, 1983. Bandyopadhyay and Ghosal, 1985), amphibia (Mallick, 1987, 1999) and bird (Joardar and Ghosal, 1977) were explored autoradiographically to pinpoint the meiotic span, the reptiles, due principally to the constrain for their laboratory rearing and acclimatization, were apparently despised. The present endeavourer is a step ahead of the initial spermatogenesis duration in the first reptile, namely *Calotes versicolor*, the common Indian garden lizard (Ghosal and Bandyopadhyay, 1983).The spermatogenesis is historically a subject of supreme fascination, fancy and sometimes fantasy not only in early medical literature but in folklores among the Indian and Babylonian soothsayers for family bereft of any successors cropping from aspermia, azoospermia etc. (Heller and Clermont, 1964). Study of duration of spermatogenesis has immense practical value under pathological and demographic conditions of man as well as veterinary animals (Bandyopadhyay,1981). A recent probe has only been made with the coming of the idea of endangered species to thrive. In consideration of this delineation reinforced with the apprehension of the decline of *Mabuyia* population, the duration of male gamatogenesis in this particular reptile was undertaken.

Materials and Method :

Experimental Animals : 27 male mature lizards (*Mabuyia carinata*), ranging in body weight from 70–100g were captured from the Burdwan University campus, West Bengal, India, during their breeding season (May-June).

Experimental Procedure : Lizards each injected intraperitoneally with 25 µ Ci of tritiated thymidine (^{3}H-TdR,specific activity 17,000 µ Ci / mM, Bhaba Atomic Research Centre, Trombay, Mumbai, India), were sacrificed at various time intervals and tissue preparations by usual squash and air-dried techniques (Sinha *et. al.*, 1983) were made between 0.32 day post injection (d.p.i) to 23.64 d.p.i. (Table 1). Tissue processing and stripping film autoradiography were done conventionally (Ghosal *et. al.*, 1983).

Since the duration of mammalian spermatogenesis is constant under normal, vesectomized, hypophysectomized and hormone treated mammals, Clermont and Harvey (1965) recommended the use of as few as six animals for this purpose. Our experience of working with submammalian species (Ghosal and Bandyopadhyay, 1983) convinces us of 20 as the number of specimens sufficient for the autoradiographic estimation of the meiotic chronology in case of any vertebrate. With this convention 27 specimens were sacrificed in this investigation.

Results and Discussion :

The debut of each labeled stages of meiosis and speermiogenesis in *Mabuyia carinata* has been portrayed in Table-I. A close look at the table reveals that labeled leptotene made its first appearance in the first four lizards (1–4) AT 0.32 d.p.i and remained radioactive upto 1.40 d.p.i with labeled zygotenes appearing for the first time at 2.28 d.p.i, it might be concluded that leptotene in *Mabuyia carinata* could be neither less than 1.40 days nor more than 2.28 days. Since the first appearance of labeled pachytene was marked at 2.60 d.p.i., the duration of zygotene could in no way exceed (2.60–1.40=) 1.20 days. The existence of pachytene as the most progressed radioactive stage upto 4.32 days and the debut of neither more than (4.56–2.28 =) 2.28 days nor less than (4.32–2.60=) 1.92 days. At 4.56 d.p.i., diplotene, diakinesis and metaphase-I were found labeled simultaneously, thereby depicting a time of transition of stages from diplotene, through diakinesis and metaphases, to spermatid to be nor more than (5.00–4.56= 0.44 day =) 11 hours. Thus quite vividly, the duration of meiosis was a little less than 5 days in this lizard.

Spermatids, undergoing spermiogenesis, were found to be the regining cohort of advanced labeled elements in the lizards sacrificed between 5.00 and 14 d.p.i. As mature labeled spermatozoa made their first appearance at 14.52 d.p.i., the phenomenon of spermatogenesis seemed to be completed sometime between (14.00–5.00=) 9.00 and (14.59–4.63=) 9.96 days. Quite prudently, meiosis and spermiogenesis in *Mabuyia carinata* are definitely completed by 14.52 days.

Although the duration of meiosis and spermiogenesis is species specific and in few cases even strain-specific, yet varies very little among genetically homogeneous animals. The mitotic cycle is accredited with the distinct divisions of the interphase stage where a G1 foreruns the S (DNA synthesis) period which is indeed antecedent to a distinct G_2 gap prior to the cell's entry into mitotic division. Unlike mitosis the premeiotic DNA synthesis has thusfar revealed no comparable gap. These form the basis of the technique employed for estimating the duration of meiosis. A comparative chronology of selected vertebrates (Table-II) emphasizes a fundamental pattern of individual duration of meiotic stages. With the compaction of zygotene (usually of less than a day duration except human male), diplotene, diakinesis and metaphase-I, the episode of pachytene and spermiogenesis are considerable prolonged. Amazingly and reassuringly enough the chronological scenario of *Mabuyia carinata* spermatogenesis is no exception and is only preceded by *Calotes versicolor* among the reptilian alles. Since pachytene is a procrastinating stage (*cf*. lampbrush chromosomes of certain salamanders), paralleling expanded diplotene (dictyotene in mammalian female), natural selection seemingly plays a paramount role in maintaining proportionate durational format of the meiotic stages in vertebrates and, may not be unlikely some subvertebrates.

TABLE 1 : Most advanced labelled stages of meiosis and spermiogenesis detected at various intervals following ^{3}H-thymidine injection into the lizards (*Mabuyia carinata*)

Most advanced remain label detected testes initially at days post injection.	Label detected initially at days post injection.	Continued to Labelled in later at the following days post injection.
Leptotene	0.32(1)*	0.80(2), 1.00(3), 1.40(4), 2.28(5)
Zygotene	2.28(5)	
Pachytene	2.60(6)	3.00(7), 4.32(8),
Diplotene,Diakinesis and Metaphase	4.56(9)	
Early spermatids	5.00(10)	6.00(11)
Mid spermatid	6.76(12)	8.00(13), 8.64(14)
Late spermatid	9.04(15)	9.72(16), 10.50(17), 11.64(18), 12.00(19), 13.00(20), 14.00(21)
Spermatozoa	14.52(22)	15.72(23), 16.72(24), 18.65(25), 20.72(26), 23.64(27)

* The number in paranthesis refers to the serial number of the lizards sacrificed.

TABLE 2 : Duration (in days) of individual stages of meiosis and spermiogenesis in selected Vertebrates as interpreted from the date of respective authors

Animals	Lepto.	Zygo	Pachy.	Diplo. to Meta-II	Spermio genesis	Total	Reference
Fish.							
Colisa fasciata	0.45	0.10	1.98	0.81	6.01	9.68	Sinha *et al*, 1979
Channa punctata	0.45	0.12	2.15	0.80	8.33	11.25	Sinha *et al*, 1983
Anabus testudineus	0.35	0.15	1.85	0.83	6.30	10.50	Sinha *et al*, 1982
Amphibia							
Euphlyctis cyanophlyctis	2.00	0.79	4.71	?	?	14.00	Mallick and Ghosal. Hamadryad. (In Press).
Rana limnocharis	1.75	0.92	2.50	0.40	4.01	10.00	Mallick and Ghosal,1999
Rhacophorus maculatus	1.88	0.40	4.72	0.66	9.82	17.25	Ghosal *et al*, 1981
Bufo melanostictus	2.27	0.76	4.48	1.88	12.67	20.00	Bandyopadhyay, 1977

Animals	Lepto.	Zygo	Pachy.	Diplo. to Meta-II	Spermio genesis	Total	Reference
Reptile							
Calotes versicolor	1.66	0.55	2.60	0.50	13.50	18.00	Ghosal and Bandyopadhyay, 1983
Mabuyia carinata	1.40	1.20	1.92	0.44	9.00	14.52	Present Investigation
Bird							
Columba livia	2.80	0.40	5.40	0.60	17.00	26.00	Joardar and Ghosal, 1977
Mammals							
Dog	4.33	0.80	15.61	1.22	22.16	42.15	Ghosal *et al*, 1983
Man	5.7	2.6	13.5	2.5	23.8	48.0	Heller and Clermont, 1963

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